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# A STUDY OF THE PROCESS OF CASEATION IN TUBERCULOSIS *

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The term caseation is applied to the gross characteristics of the necrotic material commonly seen in tuberculous infection. This necrotic tissue can be easily removed from the surrounding living tissue, leaving a cavity. It has no texture and varies in consistence from thick pus to a crumbly material not unlike cottage cheese. In these respects it differs from the necrotic tissue seen in a bland infarct or in a gumma, which cannot be readily removed from the surrounding viable tissue and which has a distinct rubber-like consistence. The necrotic material in an abscess differs from all of the above in that it is usually more liquid in character. Since these lesions are all, at times, seen in the same tissue and are either of an anemic or of an inflammatory nature, why should the necrotic tissue in tuberculosis differ from that seen in other conditions?

During the past four years an extensive study of the process of caseation has been undertaken. A large series of cases of both natural and experimental infection have been examined. Tuberculous lesions in the lung, spleen, liver, intestine, kidney, fallopian tubes and lymph nodes have been carefully studied by the serial section method. At least 200,000 sections have been carefully examined during this investigation and a large number of these sections have been examined for the presence of tubercle bacilli. Tissues from human cases obtained at necropsy and at surgical operations, from guinea-pigs inoculated with bovine and human tubercle bacilli and from fowl naturally infected with the avian strain of the tubercle bacillus have formed the basis of this study.

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Early in the study of the human tissues it became apparent that, if the natural sequence of events in the process of caseation was to be rightly understood, it would be an essential requirement to obtain tissues at known periods after infection. To obtain such tissues a series of guinea-pigs were inoculated on the same date with an equal dosage, approximately 20,000, of human tubercle bacillus, strain H37. The inoculation was made subcutaneously in the left groin. The skin over the area to be inoculated was carefully sterilized with iodine before inoculation and care was taken to avoid any contaminating infection. In none of the animals was there any evidence of acute inflammation on gross inspection at the site of inoculation at any time. Beginning with the tenth day after inoculation, animals were sacrificed every fifth day. The series gave tissues 10, 15, 20, 25, 30 and 35 days after the inoculation. Previous studies had shown that the spleen and the inguinal, iliac and par-aortic lymph nodes were the most uniformly infected following inoculation in the groin. These tissues were carefully studied for the presence of tubercle bacilli and the type of the inflammatory reaction.

## Caseation in the Guinea-Pig

Since it is possible in the guinea-pig to follow the tuberculous process, step by step, from the earliest inflammatory reaction to the stage of typical caseation, a brief account of the process as it occurs in this host will be given first. This will be followed by a description, with an attempt at correlation, of the pathologic picture as seen in natural infection in the fowl and in man.

It is now common knowledge, dating back to the work of Yersin [1] and of Borrel,[2] that when tubercle bacilli are injected intravenously for the first time in the rabbit, the first inflammatory reaction occurs within the capillaries in the various tissues and organs where the bacilli lodge. This reaction is microscopic in proportion and consists of a varying proportion of polymorphonuclear and mononuclear leucocytes. Both types of cell phagocytize the bacilli. This first polymorphonuclear leucocytic reaction is never great and is of a transitory nature, hence abscess formation does not follow. The polymorphonuclear leucocytes which have been attracted die within a few days. From this stage on the mononuclear leucocytes continue to accumulate and the tubercle is formed.

This same reaction occurs at the site of the primary inoculation in the guinea-pig. This reaction, if the size of the dose be small, is never of sufficient extent to attract attention on macroscopic examination of the site. If the dosage be large the reaction may be sufficient in amount to cause slight ulceration of the overlying tissues. The ulceration is due, apparently, to the accumulation of polymorphonuclear leucocytes with resultant small abscess formation.

The distribution of the tubercle bacilli through the body from the primary locus has been so carefully and thoroughly studied by various authors that this phase of the subject will not be entered into. Suffice it to state that the bacilli are phagocytized by both the polymorphonuclear and mononuclear leucocytes which, by their wandering proclivities, first gain access to the lymphatics and later to the blood stream.

It is in these secondary foci of infection, where the bacilli are at first few in number, that the entire and probably characteristic reaction to tubercle bacillus infection can be found. Here it is possible to follow the development of the lesion from its beginning, step by step, to caseation and to healing, if the infection is overcome. This process can be followed in the various organs of the guinea-pig. The pathologic process as it develops in the spleen is here given. The spleen is chosen for three main reasons. In the first place it is of such size that serial section of the entire organ does not entail too great technical labor. Secondly, its infection with the tubercle bacillus is entirely hematogenous and, therefore, as remote as that of any organ from the original site of inoculation. And in the third place, it has no connection with the outside world, as do the lung and kidney, and consequently would be the least liable to be involved with infectious agents other than the one inoculated.

A comparative study of tuberculosis of the spleen in the human and the guinea-pig will be reported at a later date. Here the only phase taken up will be the pathologic process as it concerns caseation.

At ten days the spleen shows no tubercle formation. By careful and persistent search one may find a single bacillus or a group of two or three bacilli about midway between the splenic artery and the periphery of the Malpighian corpuscle. These bacilli are usually within mononuclear leucocytes, although some of them are, apparently, free in the tissue. The small groups of bacilli are the ones most often found free.

From this point on one finds the greatest development of the tuberculous process in the Malpighian corpuscles, though as time elapses one also finds an increasing number of small lesions developing within the histologic unit of the spleen pulp as described by Mall.[3] There is a gradual increase in the number of tubercle bacilli up to the twenty-five day specimen. This increase is due in all probability to two factors, namely, the multiplication of the bacilli in the organ and the continued accumulation of the bacilli filtered out of the blood stream. The latter factor would appear to be the predominant one, for one finds the bacilli scattered singly or in groups of two or three, largely within mononuclear leucocytes, throughout the developing lesion. One finds the bacilli distributed in somewhat of a collar arrangement in the outer portion of the splenic corpuscle and in the histologic unit.

The cellular reaction in the fifteen and twenty day specimens consists almost entirely of mononuclear leucocytes. In many areas nearly the entire Malpighian corpuscle or the histologic unit is filled and greatly enlarged by the accumulation of these cells. This is the mononuclear or "epithelioid" tubercle as it develops in the guinea-pig. Lymphocytes are very rare in the tubercle of the histologic unit but are numerous in the area outside of the tubercle in the Malpighian corpuscle. Polymorphonuclear leucocytes are very uncommon in the tubercle but may be found in small numbers in the tissue surrounding it. There is no evidence of caseation. One does find, however, mononuclear leucocytes with vacuolated cytoplasm and very faintly staining nuclei. Mitotic figures are commonly seen in the splenic pulp and less frequently are found in the tubercle itself. There is abundant evidence that the mononuclear leucocytes migrate into the tubercle, for one very commonly finds these cells with their nuclei stretched out in a long sinuous process.

Beginning, at times in the twenty day but more marked in the twenty-five day specimen, and continuing in the thirty and thirty-five day specimens, one sees a gradual and increasing accumulation of polymorphonuclear leucocytes in the areas where caseation later becomes apparent. These areas are often near the center of the tubercle. They may, however, be eccentrically placed. They vary in size from a small focus to an area which eventually becomes as large as many tubercles. The immigration and the multiplication

of the mononuclear leucocytes by mitosis do not entirely cease but the accumulation of the polymorphonuclear leucocytes far overshadows the mononuclear infiltration, so that in many instances the inflammatory reaction resembles abscess much more than tubercle formation. Bacilli are present, more numerous in some instances and fewer in others, when compared to the fifteen and twenty day specimens. But in none of the areas where the inflammatory reaction is most intense does the typical picture of caseous material appear at this stage. Many of the smaller foci, especially in the histologic units, do show the typical picture of caseation and if the dead cells are not too closely packed together one can make out with a fair degree of certainty the outlines of the nuclei of the polymorphonuclear leucocytes. These areas also contain a varying amount of nuclear "dust." In the larger areas one sees the beginning of caseation at the periphery and in numerous instances cells in the process of disintegration. An interesting finding in these areas is the presence of mononuclear leucocytes which have ingested from one to half a dozen polymorphonuclear leucocytes. Tubercle bacilli are quite commonly found within the polymorphonuclear leucocytes which have been engulfed. Lymphocytes are rare.

In lesions of six, seven or eight weeks' duration, the picture of caseation is present in many of the larger lesions. The necrosis appears to begin at the periphery of the most intense inflammatory reaction and gradually to proceed toward the center. There is more or less solution of continuity between the viable tissue at the periphery and the necrosing inflammatory exudate within, so that a modified abscess formation is the end result.

In the inflammatory process as described, giant cells are not found. They do not appear to be an essential part of the early inflammatory reaction or of tubercle formation, at least as it is produced in the guinea-pig.

As these areas of caseation in the spleen cannot be discharged to the outside without rupture of the splenic capsule, the cellular reaction subsequent to caseation can be followed. Polymorphonuclear leucocytes are not further attracted after caseation has been produced. Mononuclear leucocytes continue to accumulate and they gradually invade the caseous mass. Lymphocytes also are attracted in increasing numbers and likewise invade the caseous material. At

this stage of the reaction giant cell formation begins to take place. The significance of this phase of the reaction will be discussed in a separate paper on giant cell formation in tuberculosis.

In many of these areas of caseation, stainable bacilli are very few or absent. In other areas one finds colonies of stained bacilli. This presents a picture quite different from the even distribution of the bacilli, individually or in small groups throughout the lesions, in the earlier stages of the tuberculous process.

Quite often the inflammatory reaction is so extensive in the larger lesions that infarction of splenic tissue not primarily involved in the tuberculous process is produced. The size of the infarction varies with the size of the artery involved, but at times involves as much as half of the organ. This point is of considerable importance in the consideration of the essential tuberculous lesion. One has, however, little difficulty in distinguishing between the infarcted non-tuberculous tissue and the areas of tuberculous involvement. In the infarcted tissue one can distinguish easily between the areas of tuberculous inflammation and the non-tuberculous splenic areas which show the outlines of the sinuses and trabeculae of the dead splenic tissue. In fact, if the infarct be large, one finds several areas of tuberculous involvement within the infarcted area. These areas can be identified with certainty by the finding of tubercle bacilli, whereas the uninvolved infarcted tissue shows no bacilli.

## Caseation in Avian Tuberculosis

Since one cannot determine the date of infection in cases of natral infection, it was impossible in the fowl to follow the development of the inflammatory reaction to the tubercle bacillus in time sequence as it was done in the guinea-pig. However, it seems logical that if stages of reaction corresponding to those observed in the guinea-pig can be found, the identity of the development of the inflammatory reaction to tubercle bacillus infection in the guinea-pig and in the fowl would be established. In the various fowl tissues studied it was possible to demonstrate numerous examples of typical mononuclear, or "epithelioid," tubercles without caseation, giant cell formation or polymorphonuclear leucocytic invasion. It was also possible to find numerous examples of tubercles without caseation but with polymorphonuclear leucocytic infiltration which

varied from a few to a large number of these cells in the areas where caseation occurred; and examples of different stages of caseation were numerous. In brief, all the stages leading up to the typical picture of caseation present in the guinea-pig were easily demonstrated in the fowl tissues naturally infected with the avian strain of the tubercle bacillus.

While it is not pertinent to go into a full discussion of the pathologic picture as seen in avian tuberculosis, there are a few facts worthy of comment. In the first place tubercle bacilli are far more numerous in the lesions than have been found in either guinea-pig or human lesions. In the caseous material large masses of bacilli in colonies are very commonly found; and in these same areas one finds large numbers of "phantoms" of unstained bacillary forms which correspond in size, shape and distribution to the stained bacilli.

In the fowl the leucocyte which corresponds to the neutrophilic polymorphonuclear leucocyte in man has an abundance of eosinophilic granules. This fact makes for easy recognition of these cells and when they are massed together in the midst of a tubercle they take an intense eosin stain which contrasts markedly to the blue-staining of the mononuclear leucocytes clustered around the periphery of the mass. The caseous material early takes a much more intense eosin stain than it does in man or guinea-pig. This fact is strongly suggestive of the important part played by the polymorphonuclear leucocyte in the production of caseous material in the fowl.

As in the guinea-pig, the polymorphonuclear leucocyte does not appear to be attracted by the caseous mass after it is once produced. In fact it is quite common to find large areas of polymorphonuclear infiltration directly adjacent to an old area of caseation with no evidence of the polymorphonuclear leucocytes attempting to invade the old caseous mass.

The mononuclear leucocyte appears to play the same rôle in the inflammatory reaction in naturally acquired avian tuberculosis as it does in the guinea-pig experimentally infected with the human or bovine tubercle bacillus. In freshly fixed tissues, one finds abundant evidence of its motility, as shown by greatly elongated nuclei. In the mononuclear tubercle, it is found to have tubercle bacilli within it. Mitotic figures in these cells are fairly easy to find in and about the tubercles.

The lymphocyte is very commonly found in the tissues surrounding the tubercle and the caseous material. It does not appear to take any active part in the formation of the tubercle or in the process of caseation.

Giant cells are usually far more numerous than in the guinea-pig tissues or in the human material I have studied.

## Caseation in Human Tuberculosis

As in the case of the fowl, the human cases were those of natural infection and no date of the initial infection could be established. It would seem that the same logic which applied to the correlation of the avian and guinea-pig tuberculous reactions would obtain in man. Stages from the mononuclear tubercle without giant cell formation, polymorphonuclear infiltration or caseation to the typical picture of caseation were found, not once but many times, in the spleen, lymph node, kidney, liver, fallopian tube and lung. So that it would appear that there is close similarity between the inflammatory reaction and the appearance of caseation in guinea-pig, fowl and man.

The caseous material in man is composed in large part of dead polymorphonuclear and mononuclear leucocytes, but it appears that the bulk of the necrotic material is made up of polymorphonuclear leucocytes. In many of the areas the inflammatory cells are so closely packed together that it is impossible to make out the outline of individual cells. The intense hematoxylin stain which these areas take during the process of cell disintegration is another fact which is evidence of the marked cell accumulation during the most active inflammatory phase. The large amount of nuclear "dust" seen in the later phase of the process can readily be explained in the same way. In the later phases of cell disintegration it is impossible to determine the types of cell which compose the compact caseous mass. But if one may judge from the earlier steps in the process the lymphocyte plays little or no part.

The cellular reaction which occurs after the process of caseation is complete, that is, when the caseous material no longer shows nuclear "dust," differs in some respects from that which takes place before caseation is produced. Around these areas one finds many mononuclear leucocytes and a varying number of lymphocytes. Both of these cell types wander into the caseous material and can

be found all the way through it if the area is not too large. Polymorphonuclear leucocytes are not present in the reaction at this time and appear to take no part in the removal or organization of the caseous material. At this stage giant cell formation begins.

Among the human tissues examined were several cases of the hyperplastic type of tuberculosis. The majority of these were in lymph nodes removed surgically. A study of the inflammatory reaction in these cases showed a variation from the process described above in several respects. Mononuclear or "epithelioid" tubercles are much more common than in the caseating type of tuberculosis. Lymphocytes are more plentiful in the tubercles, often having accumulated in considerable numbers in the small areas of necrosis of the mononuclear leucocytes and in the rare areas of typical caseation. Polymorphonuclear leucocytes are extremely rare, except where typical caseation is being produced. Areas of caseation are rare and are small when present. Fibrosis is very common, many of the tubercles showing little but hyaline collagen fibers. Giant cells are present but are not numerous. Tubercle bacilli are scarce, the finding of a single tubercle bacillus being a laborious task.

## Discussion

If the steps in the process of caseation in the guinea-pig, fowl and man as described above are a correct interpretation of the development of the tuberculous process, then it would appear that the process is the same regardless of the type of tubercle bacillus causing the infection or of the host infected. When caseation appears it is an indication of the virulence of the infection, of the lack of resistance of the host, of the dosage or of all three. When hyperplastic tuberculosis without caseation develops it is an indication of low virulence on the part of the bacillus, good resistance on the part of the host, small dosage or a combination of these factors. When caseation develops in a situation where it is possible for the necrotic material to be extruded from the body, as in the lung, kidney, skin or intestine, cavitation or ulceration ensues. If caseation occurs in a place, such as bone, where the caseous material cannot be extruded, a cold abscess will develop if the process is not successfully walled off. In case the caseous area is successfully walled off, it will either become calcified or be organized and a scar produced.

The term "mononuclear leucocyte" used throughout the description of the tuberculous process is, perhaps, not the best term to denote the cell. It is the ameboid phagocytic cell which has to do with tubercle and giant cell formation. It is apparently the same cell met with in typhoid fever, blastomycosis and other diseases and pathologic conditions where the function of phagocytosis of material that cannot be successfully handled by the polymorphonuclear leucocyte, is of vital importance. It is the cell of varied terminology. It has been designated as the epithelioid cell, the wandering mononuclear leucocyte or phagocyte, the macrophage of Metchnikoff, Evans and others, the endothelial leucocyte of Mallory, the polyblast of Maximow and the monocyte of Cunningham and Sabin. While its exact origin is perhaps still unsettled, its activities are fairly well understood. Its participation in the tuberculous process is generally accepted. A cell capable of ameboid motion, such as this cell possesses, could migrate to the site of the tubercle bacillus from the blood stream or from the surrounding tissues with equal ease. If one may judge by the presence of mitotic figures, it also multiplies after arrival at the site of infection. Whatever its name or its origin, it appears to be the greatest single factor in the destruction of the tubercle bacillus that the body possesses. It is my belief that the number of tubercle bacilli destroyed by cells of this type during tuberculous infection is but little realized.

It is the chief intent of this paper to direct attention to the rôle which the polymorphonuclear leucocyte seems to play in the process of caseation. In the mononuclear or "epithelioid" tubercle, the polymorphonuclear leucocyte is not seen until there is evidence of damage to the mononuclear leucocytes. This damage is shown by a slightly greater eosin staining of the cytoplasm, by loss of nuclear structure and oftentimes by vacuolization of the cell cytoplasm. This does not resemble caseous material. In these small areas one can often make out bacillary forms which do not stain with carbolfuchsin. This can best be demonstrated in fowl tissues where tubercle bacilli are numerous in the tubercle. Whether the polymorphonuclear leucocytes are attracted by the necrotic mononuclear leucocytes, by opsonins, by substances liberated through the death of the tubercle bacillus or by substances produced in the tissues by the multiplying bacilli is not understood.

The idea which one gains from the literature is that if the poly-

morphonuclear leucocyte is present in the inflammatory reaction to the tubercle bacillus it is there because of secondary infection. Mallory [4] states that these cells are present only when the tubercle bacilli are very numerous. He also states that caseation is due "chiefly or entirely to endothelial leucocytes plugging the lymph or blood vessels, thus cutting off nutrition" and that "the necrotic material consists largely of endothelial leucocytes." MacCallum [5] states that even in acute tuberculous inflammation "polymorphonuclear leucocytes seem to be very little attracted, their place being taken by lymphoid cells and large mononuclear wandering cells." Aschoff [6] does not mention the polymorphonuclear leucocyte in connection with caseation which he considers to be caused by the necrosis of the epithelioid cells which form the tubercle. Wells [7] quotes Schmoll as finding that caseous material, even when it breaks down and forms a cold abscess, shows much less evidence of autolysis than pus. Maximow [8] claims to have produced caseation in tissue culture where the polymorphonuclear leucocyte, because it is not regenerated in such cultures, could have but little to do with the process. He obtained in tissue culture necrotic "epithelioid" cells but it is doubtful whether this can rightly be compared to the process of caseation as it occurs in living tissues where all types of cell have free access to the site of infection. Smith [9] states that the polymorphonuclear leucocyte does not play any recognizable rôle in the tuberculous process. He also states that the appearance of the polymorphonuclear leucocyte in the early reaction to cultures inoculated intravenously is due in all probability to the suspending fluid and to disintegrating bacilli which are always present in the cultures of a few weeks' growth.

Kostenitsch and Wolkow [10] speak of a primary polymorphonuclear leucocytic infiltration which occurs a few hours up to three days after the primary inoculation of tuberculous material, and of a secondary polymorphonuclear leucocytic infiltration which occurs at the time of caseation. As tubercle formation can take place up to the stage of caseation without the polymorphonuclear leucocyte participating, it appears that the polymorphonuclear leucocyte does not in reality make a primary and secondary appearance. It is evident that there is a phase in the reaction to the tubercle bacillus where a substance is produced which definitely attracts this cell. They also state that the primary reaction is more intense than the

secondary reaction. I have found the polymorphonuclear leucocytic response much more intense after well established infection has been produced.

There is abundant evidence that the polymorphonuclear leucocyte is not attracted by caseous material after it is once formed. This can be easily demonstrated in both experimentally and naturally infected tissues. The active participation of the polymorphonuclear leucocyte in the steps leading up to caseation can be as readily demonstrated if the tissues are examined at the proper stage of the lesion. The most conclusive demonstration of this can be made in avian tuberculosis because of the easy differentiation with certainty between the polymorphonuclear leucocyte and the mononuclear elements. The eosinophilic granules in the polymorphonuclear leucocyte of the fowl allow of this sure differentiation. Although there are no eosinophilic granules in the guinea-pig or human polymorphonuclear leucocyte, these cells can be identified with certainty until they are so closely packed together in the inflammatory reaction that cell outlines can no longer be determined.

Miller [11] and others have conclusively shown the existence of reticulum which is continuous with the surrounding tissue, as a constant feature of the well formed "epithelioid" tubercle. As long as this reticulum is intact, even if death of the mononuclear leucocytes occurs, it hardly seems possible that cavitation could be brought about. During the process of caseation, however, this reticulum may be more or less destroyed and its continuity with the surrounding viable tissue broken. Because of this fact it can readily be understood why caseous material in some cases is discharged to the outside when opportunity occurs and cavitation or ulceration results. The persistence of this reticulum, even in caseous material, would also explain why in other cases cavitation or ulceration is not produced. It would seem quite probable that the destruction of this reticulum is brought about by the polymorphonuclear leucocyte with its proteolytic enzyme rather than by the mononuclear leucocyte which appears to be very closely associated with the production of the reticulum in the mononuclear tubercle.

A certain parallelism between the lipoid content of pus and of caseous material would indicate that they are probably composed largely of the same cellular elements. According to the chemical analysis of dried pus by Hoppe-Seyler,[12] the lipoid content was

21.78 per cent and 22.34 per cent in two specimens. Bossart [13] reported the lipoid content of pure caseous material as 20.75 per cent of the dried stuff. The cholesterol content varied from one-third to one-fourth of the lipoid content in the caseous material, while in pus it was approximately one-third of the lipoid content. In the dried pus there is 7.2 per cent of lecithin and in dried caseous material there is 3.83 per cent according to an analysis by Schmoll and F. Müller.[14] While the lipoid content is not identical in pus and caseous material, there is sufficient similarity to suggest that their composition is quite alike. As the ages of the pus and caseous material chemically examined without doubt were different, the caseous material being the older, it would not be surprising if the lipoid content had differed more than the analysis showed.

From the study of the inflammatory reaction early in the process of caseation in the guinea-pig, it is apparent that the polymorphonuclear leucocytes are injured before disintegration takes place. One can easily find mononuclear leucocytes which have phagocytized from one to half a dozen well formed polymorphonuclear leucocytes. Stewart, Long and Bradley [15] have recently reported this observation in tuberculin and reinfection reactions. This phenomenon appears in the areas of primary infection as well as in the reinfection and tuberculin reactions. This picture is quite comparable to the phagocytic action which the mononuclears display for lymphocytes and erythrocytes in typhoid fever. This early injury to the polymorphonuclear leucocyte might explain why autolysis and softening do not take place in the process of caseation as in an ordinary abscess. Several investigators have failed to find any evidence of autolytic ferments in caseous material. Jobling and Petersen [13] suggest that this inhibition of autolysis is due to the presence of unsaturated fatty acids derived from the tubercle bacillus. As the autolytic ferment of the polymorphonuclear leucocyte requires an alkaline medium to enable it to bring about liquefaction, it would seem plausible that an acid reaction might be the cause for the failure of the leucocyte to bring about liquefaction as it does in a pyogenic infection. It would seem that some such phenomenon rather than a marked difference in the cellular content of caseous material and pus would be a logical explanation for the difference between caseation and abscess formation.

## Conclusions

Caseation, cavitation and ulceration in tuberculosis appear to be due to the active participation of the polymorphonuclear leucocyte in the inflammatory reaction to the tubercle bacillus.

The process of caseation appears to be the same in naturally acquired tuberculous infection in fowl and man and in laboratory animals experimentally infected.

The presence of the polymorphonuclear leucocyte in tuberculous inflammation is not an indication of secondary infection with pyogenic bacteria. It is an indication of the production by the growth or death of the tubercle bacillus or by the action of the tubercle bacillus upon the tissue in the tubercle, of a substance which strongly attracts the polymorphonuclear leucocyte.

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## EXPLANATION OF PLATES

### Plates 55–61

Fig. 1. Guinea-pig spleen. Mononuclear tubercle in histologic unit of the pulp. Twenty days after groin inoculation. The tubercle is composed of mononuclear leucocytes, probably of reticulo-endothelial origin. In such a tubercle lymphocytes and polymorphonuclear leucocytes are extremely rare. No caseation. x 350.

FIG. 2. Guinea-pig spleen. Twenty-five days after groin inoculation. This tubercle is in the same anatomic position as the one in Fig. 1. Note the considerable number of polymorphonuclear leucocytes in the central portion. Lymphocytes are rare. There is no evidence of caseation. x 350.

FIG. 3. Guinea-pig spleen. Thirty days after inoculation in the groin. The lesion is in a histologic unit of the pulp. The dark mass is composed very largely of polymorphonuclear leucocytes. Lymphocytes very rare. No evidence of caseation. x 350.

FIG. 4. Guinea-pig spleen. Thirty-five days after groin inoculation. The lesion is in the outer portion of a Malpighian corpuscle. The dark mass is made up largely of polymorphonuclear leucocytes. Caseation is beginning as is shown by the hazy appearance of the tissue at the periphery. Lymphocytes are present in small numbers in the tissue around the caseating area. x 350.

FIG. 5. Guinea-pig spleen. Thirty-five days after groin inoculation. The lesion is in a histologic unit of the pulp. There is considerable nuclear "dust" in the center with typical caseation in the periphery. Note the predominance of mononuclear leucocytes around the area in the periphery of the caseous material. Several of these cells show elongated nuclei, presumably in the process of migrating into the caseous area. Lymphocytes are more numerous than in the preceding figures. Polymorphonuclear leucocytes while present are few in number. x 350.

FIG. 6. Avian tuberculosis. Typical mononuclear tubercle in turkey liver. Natural infection. Acid-fast stain. Corresponds to Fig. 1. x 350.

FIG. 7. Avian tuberculosis. Natural infection. Spleen of chicken. The dark staining cells are polymorphonuclear leucocytes. There is beginning caseation. x 350.

FIG. 8. Avian tuberculosis. Natural infection. Spleen of chicken. More marked accumulation of polymorphonuclear leucocytes than Fig. 7. This corresponds to Fig. 3. There was no evidence of caseation in the entire lesion which was serially sectioned. x 350.

FIG. 9. Avian tuberculosis. Natural infection. Liver of chicken. The dark area in the center is a compact mass of polymorphonuclear leucocytes. There is beginning caseation at the periphery. Note the vacuolated mononuclear leucocytes surrounding the area of polymorphonuclear leucocytes. This lesion corresponds to Fig. 4. x 350.

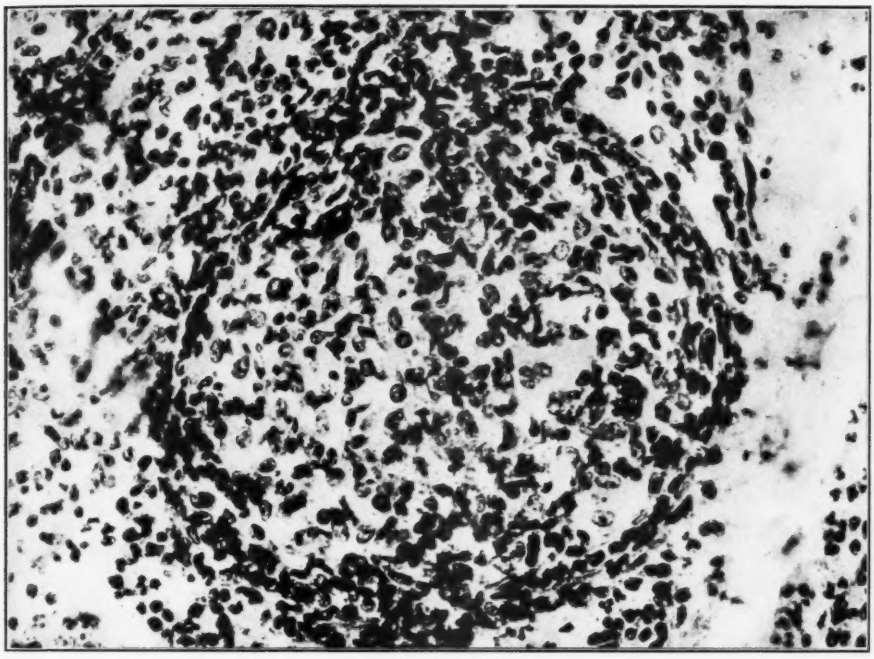
FIG. 10. Avian tuberculosis. Natural infection. Turkey liver. The lesion to the left shows old caseation and corresponds to Fig. 5. The lesion to the right shows a much greater accumulation of polymorphonuclear leucocytes but is about the same stage as Fig. 9. The polymorphonuclear leucocytes do not appear to be attracted by the older caseous material. Note the sharp line of demarcation between the two lesions. x 100.

FIG. 11. Human tuberculosis. Human spleen. Mononuclear tubercle. There is no caseation. Note the zone of polymorphonuclear leucocytes in the periphery of the tubercle. x 200.

FIG. 12. Human tuberculosis. Fallopian tube. Compare with Fig. 3. Note the elongated polymorphonuclear and mononuclear leucocytes, presumably migrating into the tubercle. There is no evidence of caseation. Lymphocytes are very rare. x 350.

FIG. 13. Human tuberculosis. Lymph node. The edge of a large lesion corresponding in age to Fig. 4. There is a lymphocytic zone above, a mononuclear zone in the middle and polymorphonuclear leucocytes below. Compare the nuclear shapes below with the nuclei of the polymorphonuclear leucocytes in Fig. 12. X 350.

FIG. 14. Human tuberculosis. Kidney. A small area of caseation. Compare with Fig. 5. Note the mononuclear leucocytic zone around the area of caseation, the nuclear "dust" in the area of caseation and the rarity of polymorphonuclear leucocytes at this stage. X 350.



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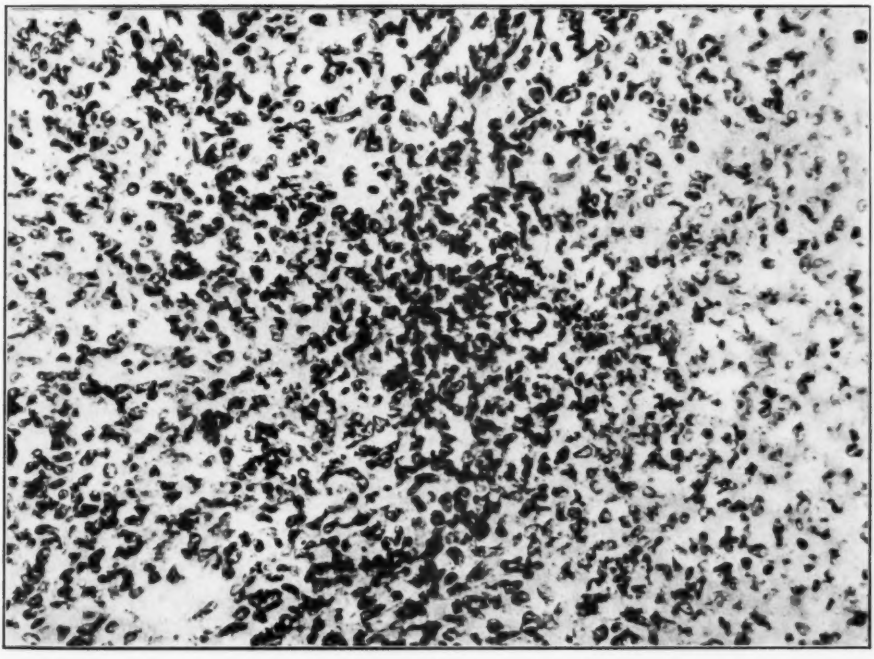

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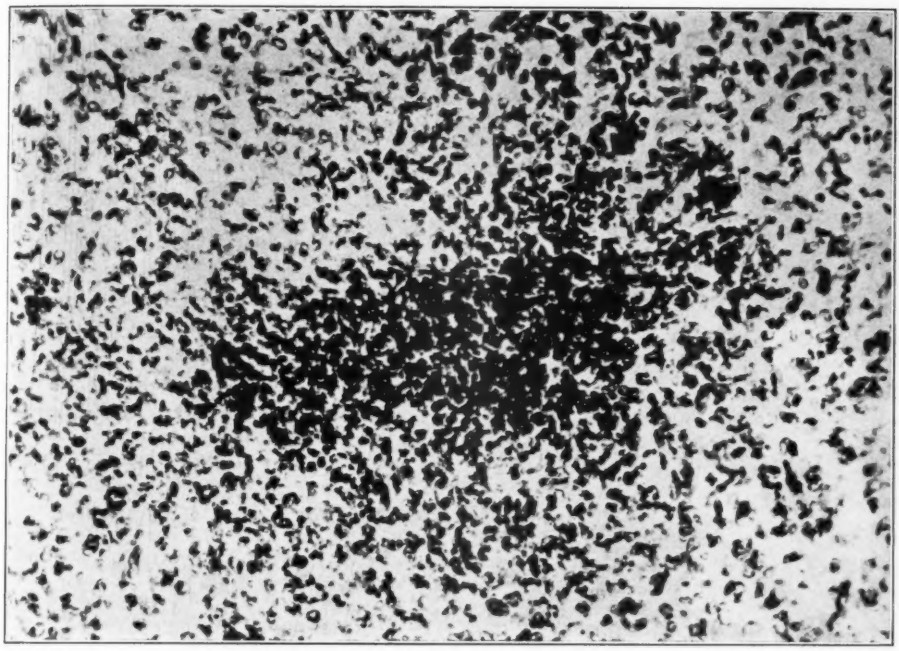
Medlar Caseation in Tuberculosis



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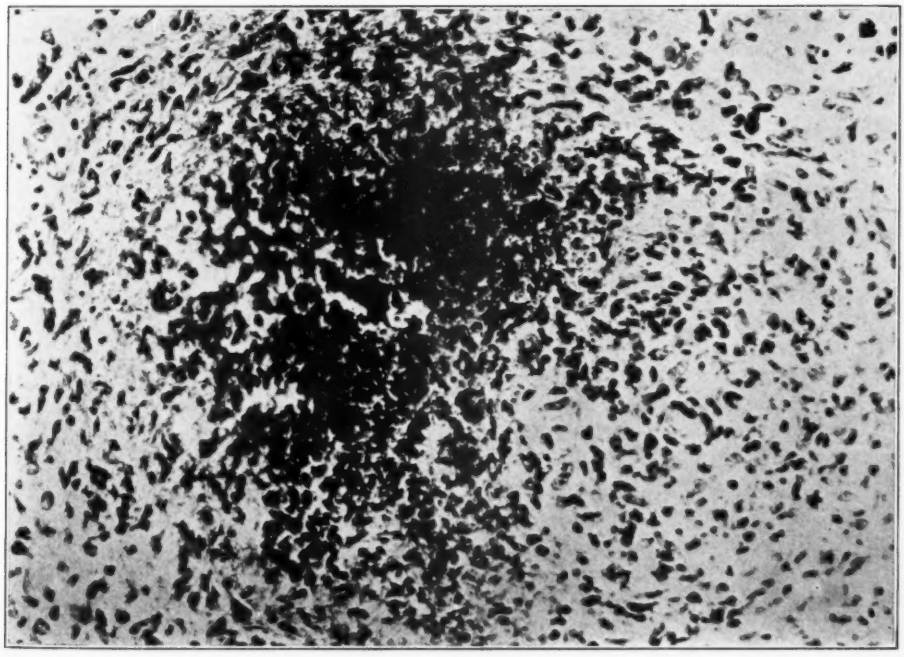

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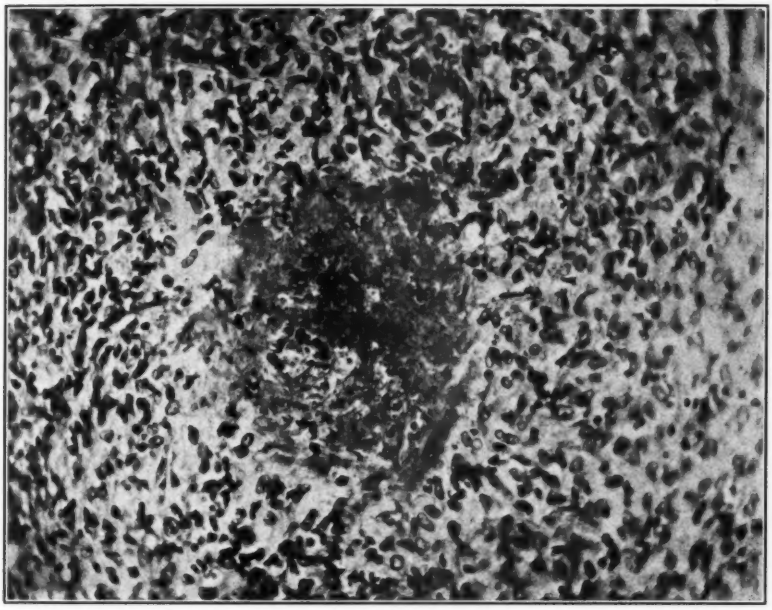

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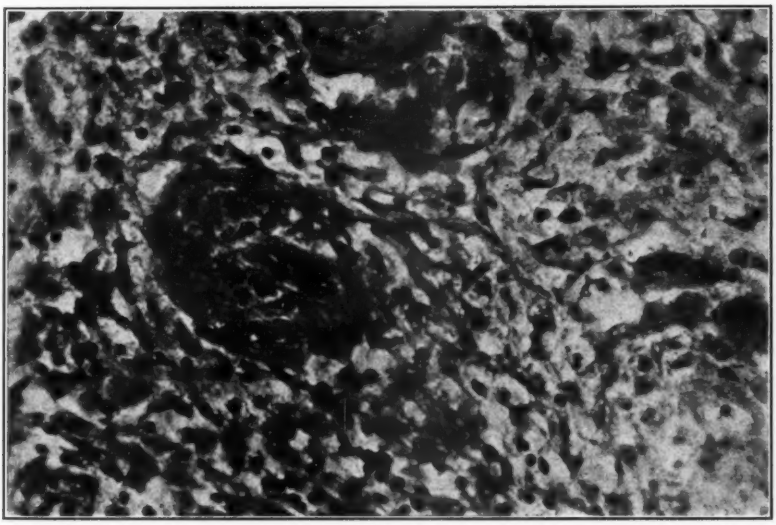

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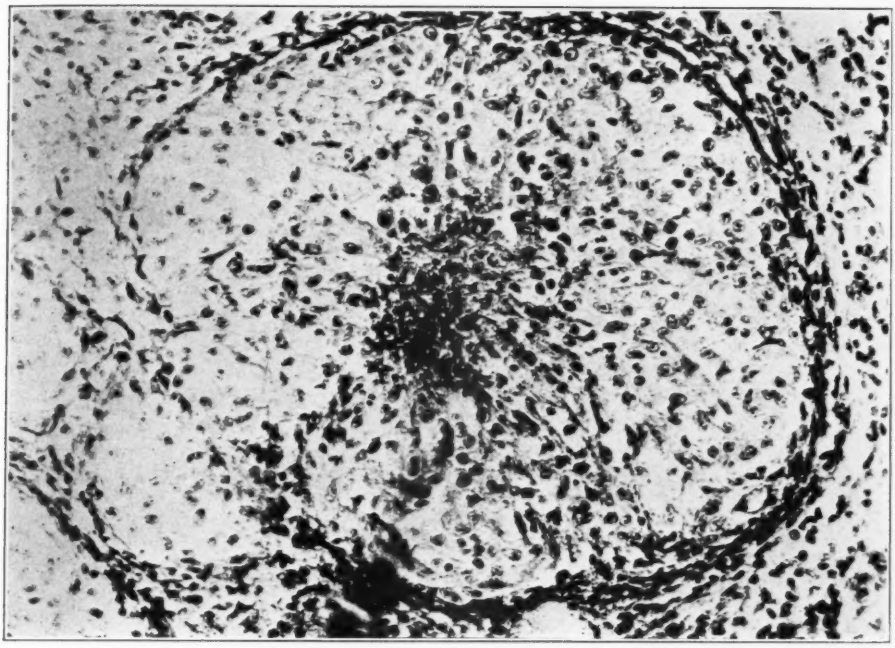

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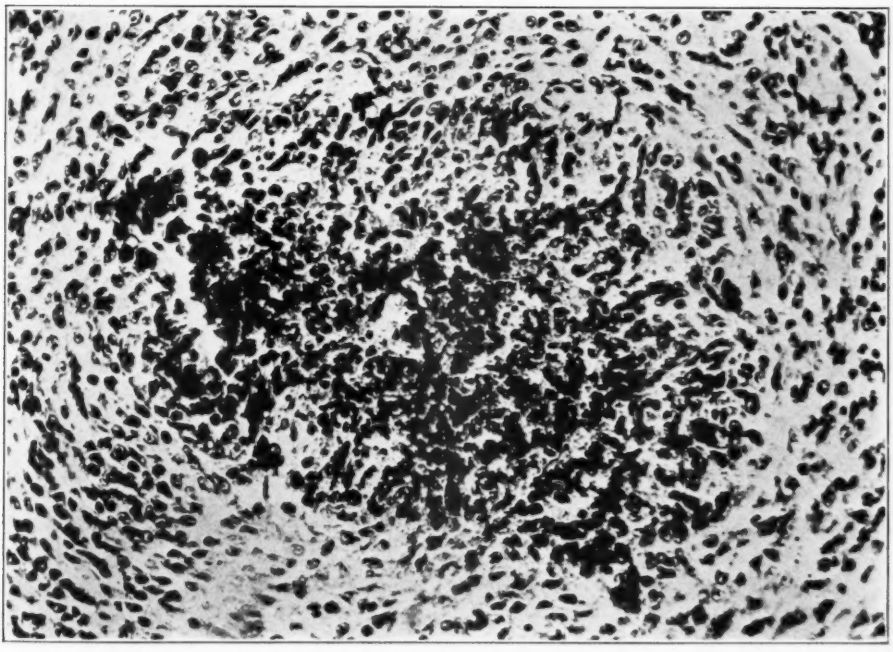

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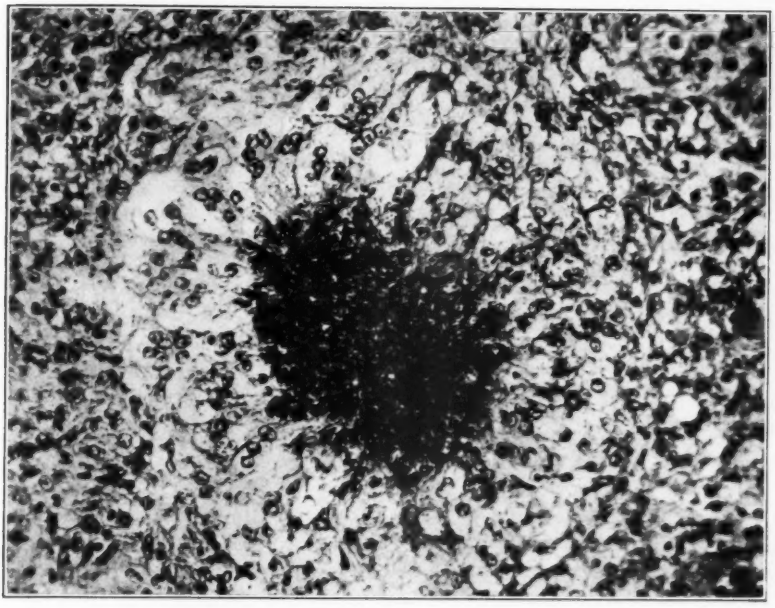

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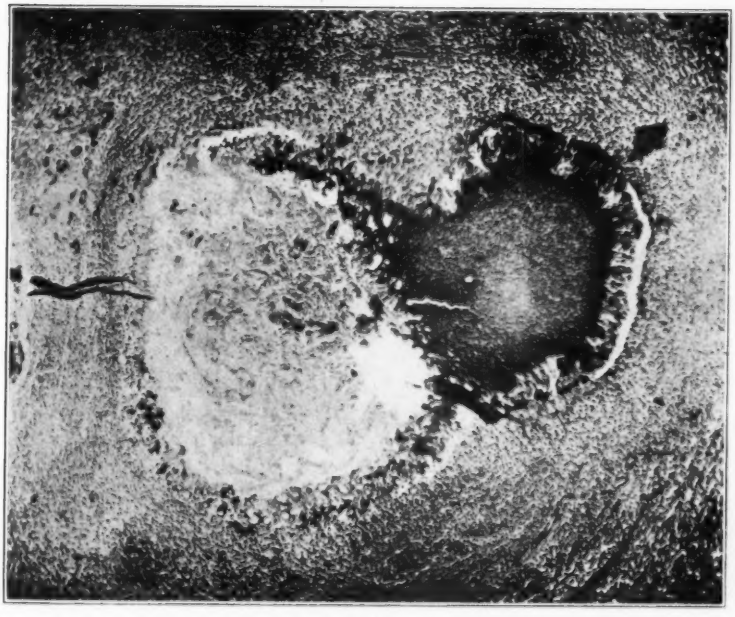

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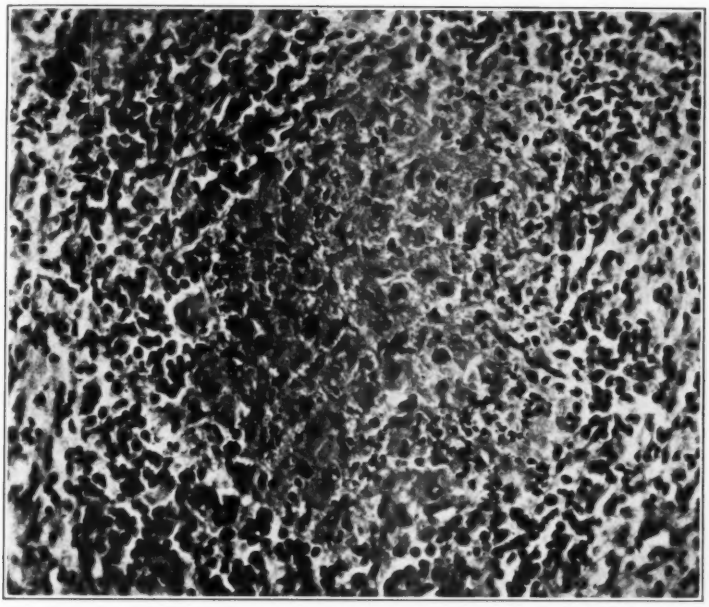

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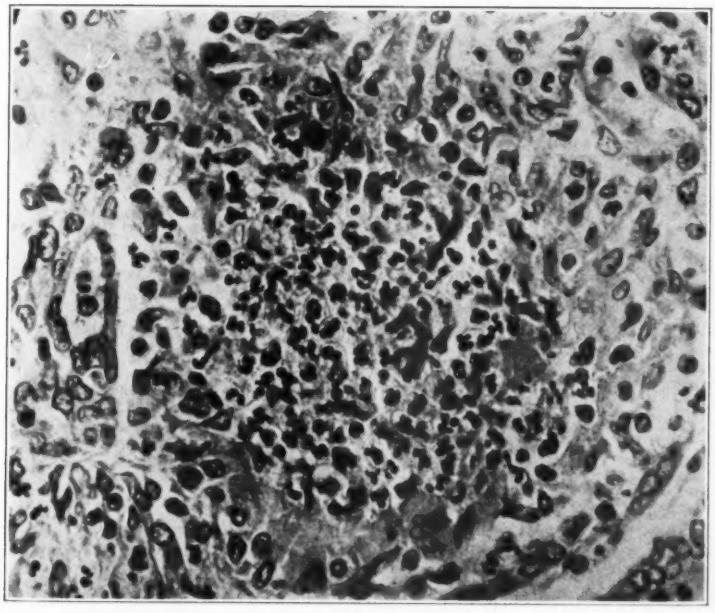

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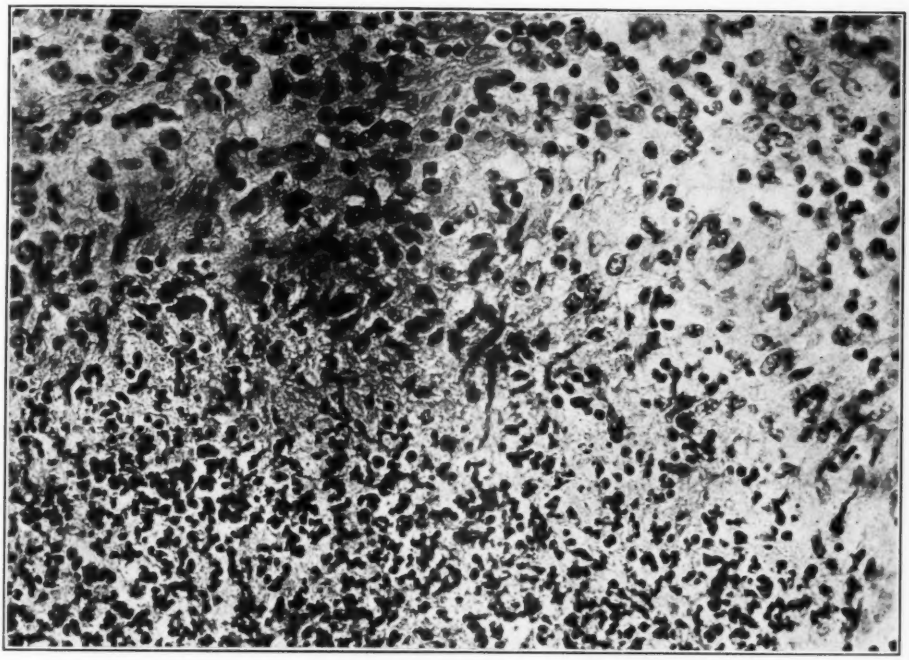

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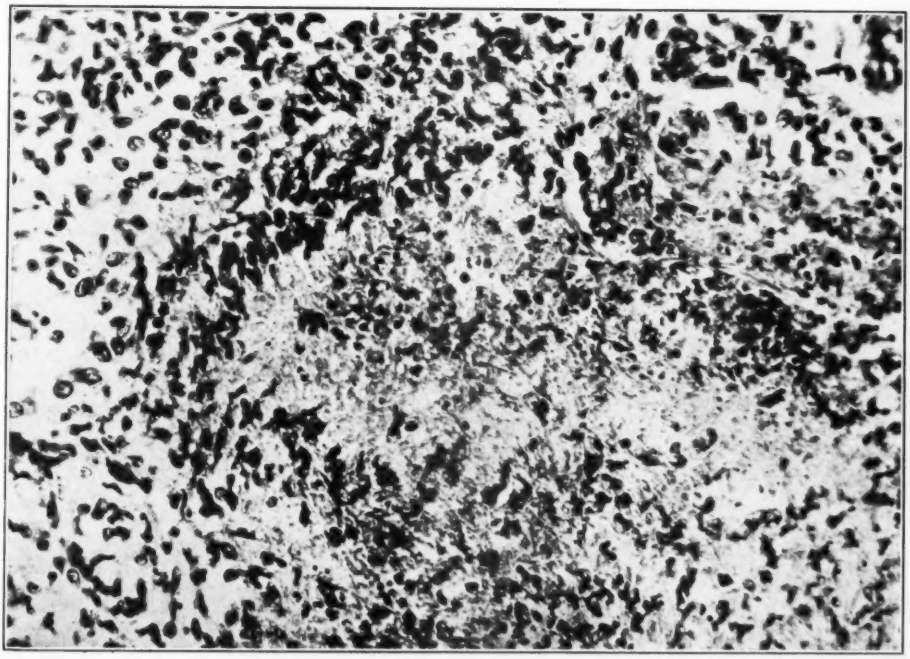

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Medlar Caseation in Tuberculosis

# GIANT CELLS AND THEIR RELATION TO CASEATION IN TUBERCULOSIS *

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The presence of giant cells, especially of the Langhans type, together with mononuclear tubercles is considered sufficient evidence in tissues to warrant the diagnosis of tuberculosis, even if tubercle bacilli are not demonstrated. Such cells are quite generally considered to be different from the ordinary foreign body giant cells and hence peculiar to tuberculosis. The frequent appearance of this cell in the midst of a tubercle, often occupying almost the whole of the structure, has led to the designation of such a lesion as a giant cell tubercle. To understand the significance of this cell mass in the tuberculous reaction, it would appear that two facts should be established. The first fact is the period in the tuberculous reaction in which the cell is produced, and the second is the mode of its formation and constitution.

The material which formed the basis of this study is a large number of serially sectioned tissues from guinea-pigs experimentally infected with human and bovine bacilli, fowl naturally infected with the avian bacillus and human tissues from necropsies and surgical operations. Serial sections were found of great value for two reasons. It was possible to examine the entire lesion and to reconstruct the giant cells, thus giving a more comprehensive idea of the size, shape and general characteristics of these cell masses.

## GIANT CELL FORMATION IN THE GUINEA-PIG

Perhaps the most striking feature in the developing cellular reaction to the tubercle bacillus is the absence of giant cells. The next feature of prominence is the very common occurrence of caseation. From this it would appear that these cells are not essential in the process of caseation. This feature is of prime importance in the

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understanding of the rôle of this cell in the tuberculous reaction, for in the guinea-pig we have a host of high susceptibility to tubercle bacillus infection.

In the guinea-pig the mononuclear leucocyte plays the chief rôle in the production of the tubercle, the polymorphonuclear leucocyte in the production of caseation and the mononuclear leucocyte and lymphocyte in the disposing of the caseous material. It is in this later phase of the reaction, the appearance of caseation, that giant cells appear. I have not observed such cell formation in the earlier phases of the inflammatory reaction. When they do appear, one finds them in areas where the amount of necrosis or of caseation has been small and not in the areas where more extensive caseation has developed. It would seem that the ground substance or matrix of these cells is a small area of necrotic or caseous material. The peculiar arrangement of the nuclei in the periphery of the cell appears to be due to the immigration of the mononuclear leucocytes into the periphery of the dead tissue. One very commonly finds mononuclear leucocytes with markedly elongated nuclei, suggesting that they are on the point of entering the necrotic area. Lymphocytes are quite often found among the mononuclear leucocytes and they also appear in elongated form. Polymorphonuclear leucocytes are extremely rare in these areas. When mononuclear leucocytes accumulate in sufficient numbers, the picture of a giant cell tubercle is produced.

### Giant Cell Formation in the Fowl

As the cases of avian tuberculosis were all natural infections, it was not possible to follow in time sequence the development of the tuberculous reaction. It was possible, however, to find numerous examples of the various stages as seen in the guinea-pig. Giant cells were far more numerous than in the guinea-pig. It was apparent that here we were dealing with a much better balance between the resistance of the host on the one hand and the virulence of the parasite on the other.

The same cell types react in the tuberculous process in the fowl that do in the guinea-pig in the formation of the tubercle and in caseation. It would appear that the giant cells would in all probability be formed in the same manner. In fact, the fowl tissues show much more strikingly the process of such cell formation than do the

tissues of the guinea-pig. Numerous examples of giant cell tubercles were found. In many instances it was possible to demonstrate the presence of cell exudate undergoing caseation in the center with large numbers of mononuclear leucocytes in the periphery of the caseating material. The intense eosin staining of the caseating mass makes a striking color contrast with the hematoxylin-staining mononuclear leucocytes surrounding it. Many of the isolated giant cells were roughly the size of mononuclear tubercles in the same field. Mononuclear leucocytes were numerous in the tissue adjacent to the caseating material and there was evidence that they wander into it. Mitoses in these cells were quite often found. Lymphocytes were also rather numerous among the mononuclear leucocytes. In the giant cell, where caseation has progressed to such a point that no nuclear fragments stain with hematoxylin, polymorphonuclear leucocytes were no longer found. In the earlier phase polymorphonuclear leucocytes were present among the mononuclear leucocytes at the periphery of and in the necrosing area.

Another finding of interest in the fowl tissues was the presence of from two to a dozen or more giant cells in close proximity to each other. In some of these areas there were bits of caseous material in the central portion which showed no nuclear content. It would seem that these areas were originally relatively small areas of caseation which for some reason have become broken up into smaller units. These units have then been surrounded and invaded by mononuclear leucocytes, thus forming giant cells. On tracing these areas through in serial sections, one found that many of these cell masses were continuous with an area of caseous material which might not contain any nuclear structures.

With an acid-fast stain large numbers of bacilli could be demonstrated in many of the giant cells. They occurred in colonies and in scattered groups, both in the necrotic area and in the nuclear zone. In these same cell masses there was present a small to a large number of clear spaces which corresponded in size, shape and distribution to the stained bacilli. In other giant cells no stained bacilli were found, but "phantoms" of bacillary forms were very commonly present.

## Giant Cell Formation in the Human

It is possible to demonstrate in suitable human tissue all of the stages of the tuberculous reaction as seen in the guinea-pig or in the fowl. It seems logical, therefore, to expect that giant cell formation would occur in the same manner.

In the examination of old and of more recent tuberculous lesions in the same case one commonly finds giant cells in the former and not in the latter. From this it would appear that the formation of these cells is definitely associated with the more chronic lesions where a better balance between infection and resistance has been established.

These cells are but seldom found in close relation to large areas of caseation. They are found at a variable distance from the caseous mass and in this location one also finds giant cell tubercles.

As far as can be determined this process does not occur during the formation of the "epithelioid" tubercle. Neither does it appear to play any part in the process leading up to caseation. Apparently it does not occur in the tubercle unless necrosis or caseation supervenes. This is well illustrated in certain cases of hyperplastic tuberculosis where there is little evidence of necrosis or of caseation. In such cases giant cells are extremely rare. In other words, it seems that this cell is a late product in the tuberculous reaction, if it occurs at all.

If one studies a large number of giant cells, the variation in size, shape and nuclear content is very striking. The arrangement of the nuclei also shows great variation, although the peripheral arrangement is perhaps the most common. The substance which has been termed the cytoplasm of the cell mass apparently bears very little relation to the nuclear content, for one finds some cells having few nuclei with a larger amount of so-called cytoplasm than others which are heavily loaded with nuclei. By rough estimation of the volume of the cytoplasm of mononuclear leucocytes and of giant cells, computed from serial sections, one finds such great variation that it would seem highly improbable that the cytoplasm of the multiplied or accumulated mononuclear leucocytes alone constitutes the cytoplasm of the cell.

These cells vary from 30 to 300 microns in thickness. Reconstruction shows them very irregular in outline and more or less spheroid

or lenticular in shape. What appears to be two well formed and separate cells in one section often becomes a single larger cell a few sections farther on. This union and division may be present in more than one level. When reconstructed, some of these cells have the form of a capital letter H.

The distribution of the nuclei in the various levels of the giant cell is just as variable as the shape and size. The nuclei may be all through the cell at the center and around the periphery a few sections away, or the distribution may be reversed. The number of nuclei varies from less than 100 to more than 1,000 in the entire cell mass. Two types of nuclei are often present, those of the mononuclear leucocyte, which predominate, and those of the lymphocyte, which are present in small numbers. Both of these types of cell with markedly elongated nuclei can be found entering the cell mass. Very rarely a polymorphonuclear leucocyte is also seen in the giant cell.

In cases of pulmonary tuberculosis associated with a considerable degree of anthracosis in which the tuberculous process is of such a character that giant cells are common, it is quite often possible to find them containing anthracotic pigment. In such cells the pigment is peripherally arranged if the nuclei are so arranged and is scattered throughout the cell if the nuclei are thus placed. If the nuclei are peripherally arranged, the central portion of the cell is pigment-free. The amount of pigment varies from a few granules to an amount which, even under low power microscopic examination, gives the cell a distinct brownish black color. Instances have been observed in which the only pigment present has been in association with a single nucleus considerably separated from the major number of closely grouped nuclei in the cell. In the tissue surrounding the pigment-containing giant cells, pigment-laden mononuclear leucocytes are commonly encountered. From these observations it would seem fairly certain that the pigmentation of these cells has been brought about by the invasion of dead tissue by one to many pigment-containing mononuclear leucocytes.

These findings led to a more careful study of the substance which formed the matrix of the cell mass. It was found that the condition present in the human tissue corresponded to that observed in the guinea-pig. In other words, in order to have the formation of such cells brought about in human tuberculosis it is necessary to have

small areas of caseation, or of necrosis of the mononuclear leucocytes, produced at a previous date.

Since reticulum is a constant finding in a well formed "epithelioid" tubercle and since it appears that giant cells are formed in such tubercles after caseation or necrosis has supervened, it became of importance to determine the relation of reticulum to these cells. Tissues for this study were very kindly put at my disposal by Dr. William Snow Miller. This study revealed varying amounts of broken, jagged particles of disintegrating reticulum in practically all parts of the cell. In most places the cell was entirely separated from the surrounding well formed reticulum but in some areas these fibers extended into the cell mass for a short distance. The even texture of the viable reticulum stood out in sharp contrast to the jagged and granular bits of reticulum scattered haphazardly within the giant cell.

## Discussion

A comprehensive review of the literature on giant cell formation will not be undertaken here. Suffice it to say that at the present time there are two theories. There is the original conception of Weigert that such cells are formed by the continued nuclear division, without division of the cytoplasm, of a single cell. This theory still has its ardent supporters, among whom are Cunningham, Sabin *et al.*[1] These authors would have this cell formed by continued amitotic division of the monocyte. The other theory is that the cell is formed by the fusion of many "epithelioid" cells. The most recent advocate of this theory is Maximow,[2] who would have the lymphocyte also participate in this fusion.

It is generally considered that giant cell formation precedes caseation and that it becomes involved in and destroyed by this process.

The findings presented above would seem to place in a different light the time and mode of production and the function of the so-called giant cell. It appears quite certain that this formation does not precede small areas of caseation or of necrosis. This would place the cell late in the tuberculous reaction, as far as the individual lesion is concerned. It would place such a cell in the reparative stage of the disease. Thus one can account for the inability to demonstrate tubercle bacilli in many of these cells.

Placing the giant cell in the reparative stage of the disease would

explain the finding of solitary giant cell tubercles in tissues which show no other evidence of tuberculous involvement. It coincides, in a way, with the statement of Smith [3] that "the occasional single giant cell in otherwise normal tissue of tuberculous cattle may be regarded as a tubercle in its simplest as well as most highly immune type." I do not consider this to be the simplest or the most highly immune type of tubercle, but rather a tubercle which has undergone necrosis or caseation, probably the latter, and is in a state of repair after the damage has been done. I believe the simplest and most highly immune type to be the mononuclear or "epithelioid" tubercle in which tubercle bacilli are destroyed without necrosis ensuing and without giant cell formation. The healed stage of such a tubercle would be, at most, a minute scar.

The finding of both lymphocytes and mononuclear leucocytes in the giant cell corresponds to the cellular reaction in the larger areas of caseation where both types of cell are found penetrating the caseous mass. It would appear that these larger areas of caseation prevent formation of giant cells because of their size and not because of any difference in cellular reaction. Definite evidence has been presented in the fowl, and the same picture on a smaller scale has been observed in the human and in the guinea-pig, that if the caseous material is broken up into smaller units, groups of giant cells will be produced. This evidence would suggest that a large number of these cells have caseous material as their matrix, and that this constitutes in large part the substance which has been regarded as the living cytoplasm of the cell. As caseation often takes place in the central portion of the tubercle, this would also account for the presence of such a cell in this location.

The finding of fragmented, jagged bits of reticulum and of anthracotic pigment in various parts of giant cells adds weight to the above evidence.

Further corroborative evidence is the finding of mononuclear leucocytes and lymphocytes wandering into the dead substance. Why the cells should not penetrate farther into the dead tissue in the case of the Langhans type of cell, is not clear. It may be due to lack of chemotaxis, but it would be more likely that the compactness of the material impedes further progress. The lack of compactness would explain why the invading cells are present in all portions of the dead tissue in some of the areas.

The theory of giant cell formation from a single cell seems to be incompatible with the evidence cited above. Whether the cells fuse after they wander into the dead tissue seems impossible of determination. Whether they do or do not fuse would appear to be of minor importance. The presence of lymphocytes in the cell can be explained on a basis other than that they are in process of being transformed into mononuclear leucocytes. No evidence is at hand, in the tissues examined, to suggest any such transformation.

The function of the giant cell, which from the above evidence appears not to be a true giant cell, apparently is to digest or remove in some manner the dead tissue. After this has been accomplished all evidence of the process left would be a scar which could no longer be identified as a lesion peculiar to tuberculosis.

It would seem that a true giant cell would be one where there is, when compared to a normal sized cell, a large amount of living cytoplasm with one or many living nuclei. Dead material would constitute no part of such a cell. Such giant cells are those seen in the most malignant types of neoplastic growths. With this as a standard, the cell masses in tuberculosis can hardly be looked upon as true giant cells.

## Conclusions

Giant cell formation in avian, bovine and human tuberculosis appears to be brought about in a similar manner.

Giant cells are an indication of a reparative process in small areas of caseation or of simple necrosis of tissue — a reaction to a foreign body.

Giant cells in tuberculosis are not true giant cells. They are bits of dead inflammatory tissue which have been more or less completely surrounded and invaded by mononuclear leucocytes, less commonly by lymphocytes and very rarely by a polymorphonuclear leucocyte.

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2. Maximow, A. A. *J. Infect. Dis.*, 1925, xxxvii, 418.
3. Smith, T. *J. A. M. A.*, 1917, lxviii, 764.

## DESCRIPTION OF PLATES

### PLATES 62–65

FIG. 1. Guinea-pig spleen seven weeks after groin inoculation. Small area of caseation with four giant cells. Lesion in histologic unit of the pulp. x 350.

FIG. 2. Chicken spleen. Massing of mononuclear leucocytes around an area undergoing caseation. Numerous polymorphonuclear leucocytes in center still intact. x 350.

FIG. 3. Chicken spleen. An area similar to Fig. 2, except that the mass in the center has all undergone caseation. Note mitotic figures at about 6.15 and 7.30 o'clock. x 350.

FIG. 4. Turkey liver. A "nest" of giant cells. This represents an area of old caseous material which has broken up and in which the invasion of mononuclear leucocytes has caused giant cells to be formed. Note that the caseous material in the center is devoid of nuclei but otherwise resembles the cytoplasm of the giant cells. x 350.

FIG. 5. Two giant cells in human spleen. Both show lymphocytes as well as mononuclear leucocyte nuclei. At the lower right hand corner of the cell to the right there is a mononuclear leucocyte elongated in the process of entering the giant cell. x 200.

FIG. 6. Giant cell in human lung. The cytoplasm is composed of caseating material with much nuclear "dust" present. Adjacent to the mononuclear nuclei at the lower edge are a few anthracotic granules. x 350.

FIG. 7. Giant cell in human fallopian tube. Note lymphocytes in center and two elongated mononuclear leucocytes at the lower right hand corner. x 500.

FIG. 8. Giant cell in human lung. Note anthracotic pigment scattered in region of nuclei. Also individual anthracotic-laden mononuclear leucocytes in the tissue above and below the giant cell. x 350.

FIG. 9. An area of caseation in a human lymph node. Two well formed giant cells at the left and one forming at the right. Mononuclear leucocytes and lymphocytes are invading the area in the center. x 200.

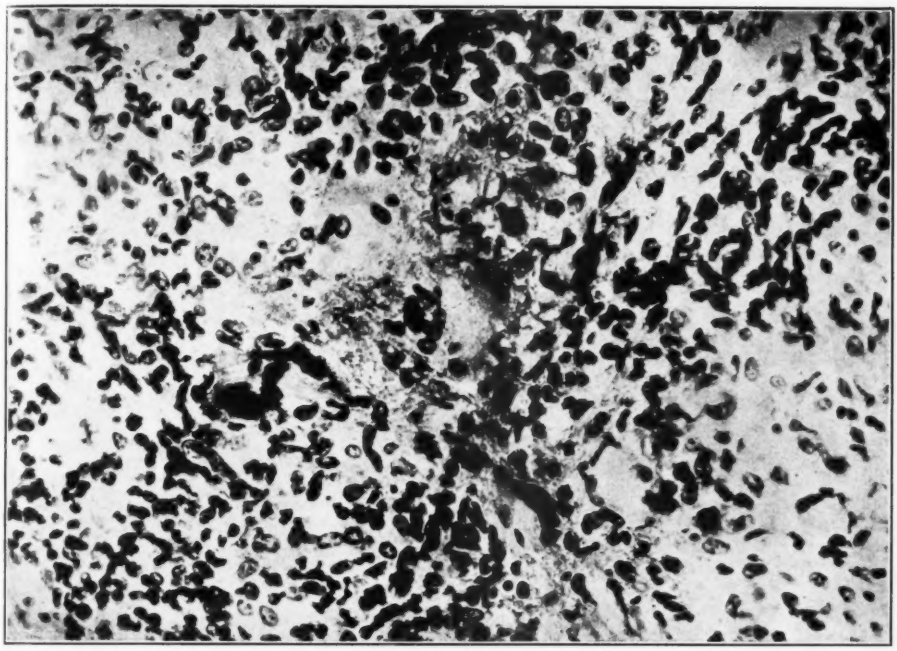
FIG. 10. Giant cell tubercle in human kidney. Note the arrangement of the nuclei about four centers. x 200.

FIG. 11. Human lung showing giant cells in various stages of formation. x 100.

FIG. 12. Giant cell at right of Fig. 11. Reticulum stain. Note the small fragments of reticulum in the center. x 500.

FIG. 13. Human lung. Reticulum stain. Mononuclear tubercle with basketwork of reticulum and no necrosis below. Compare size of giant cell above with tubercle below. Note separation of reticulum from giant cell at its periphery and the particles of jagged reticulum within the giant cell. x 500.

FIG. 14. Human lung. Reticulum stain. Giant cells showing broken reticulum within them and separation from surrounding viable reticulum. x 500.



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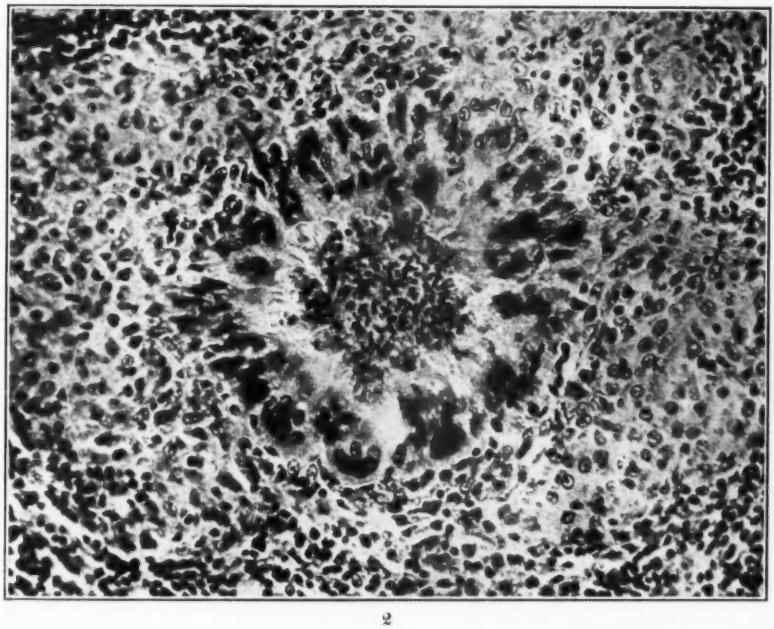

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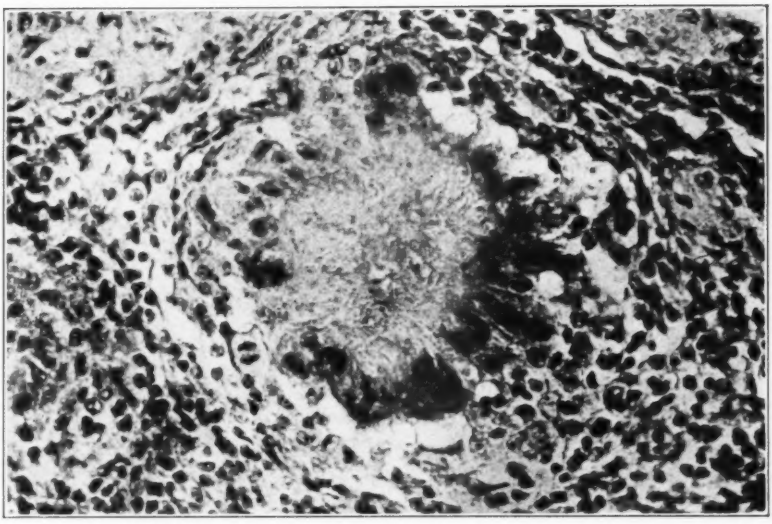

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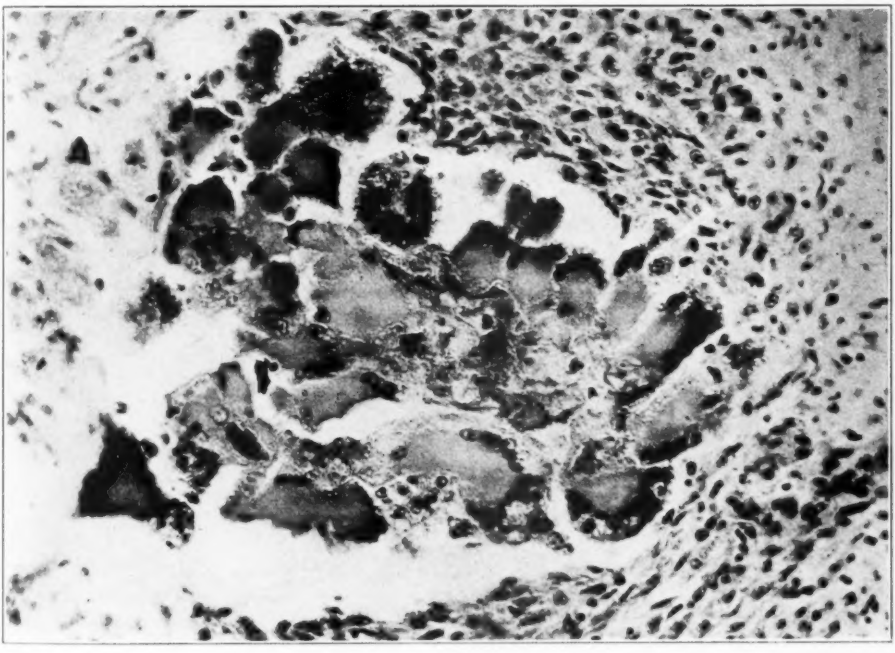

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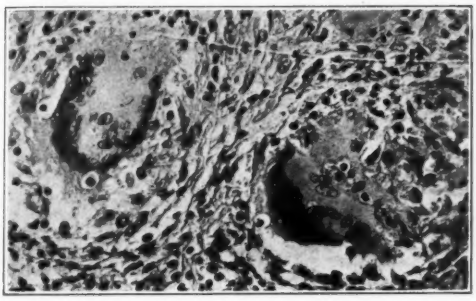
Medlar Giant Cells and Their Relation to Caseation



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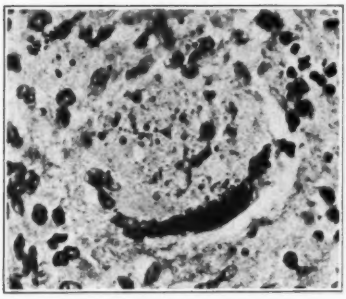

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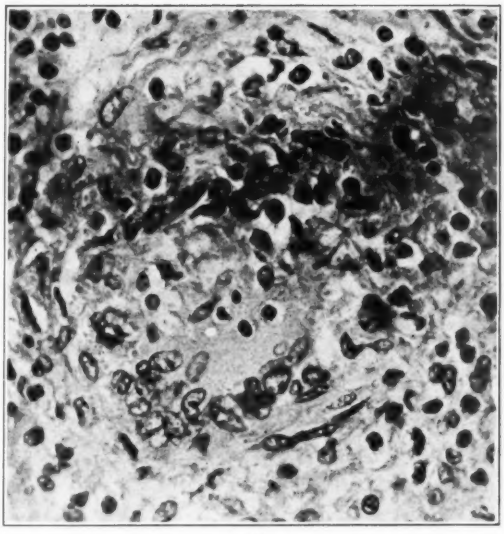

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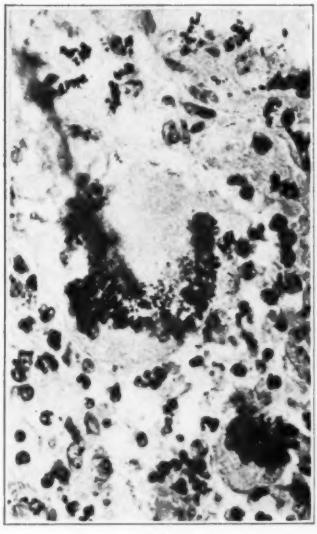

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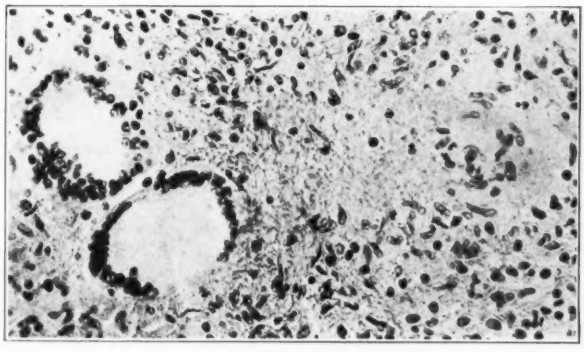

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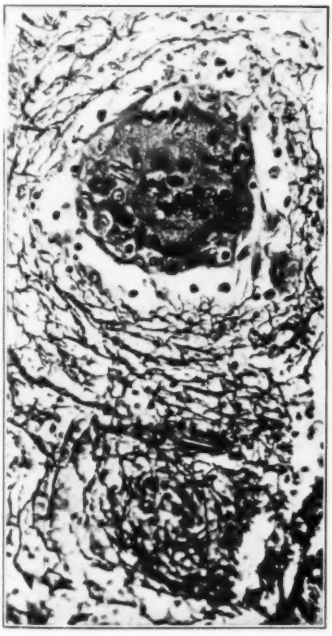

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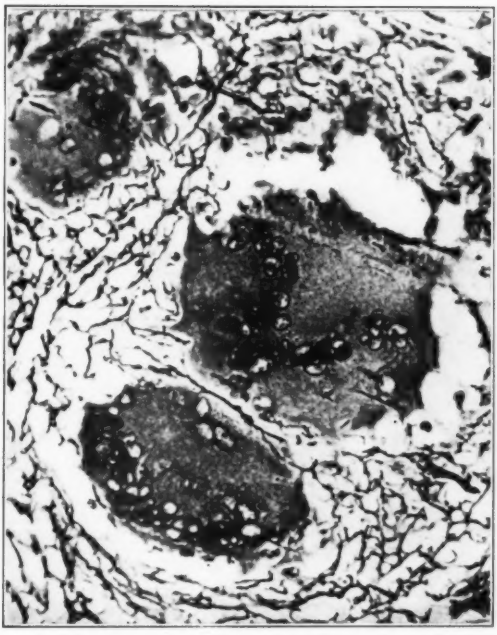

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Medlar Giant Cells and Their Relation to Caseation

# AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF THE THYROID GLAND IN THE RAT

## WITH SOME OBSERVATIONS ON TRANSPLANTATION OF THE PARATHYROID, UTERUS AND OVARIES *

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In former papers we compared auto- and homoiotransplantation of thyroid and other tissues in the guinea-pig. It was then of interest to determine how far the conclusions we arrived at in the case of the guinea-pig hold good also in the case of other animals and how far we have to deal with reaction types that apply generally. For purposes of comparison, therefore, we carried out auto- and homoiotransplantations in the rat. On a former occasion we had already compared multiple transplantations of organ pieces in the rat under conditions of homoio- and syngenesiotransplantation.[1]

### A. AUTOTRANSPLANTATION

In the first two weeks following transplantation, conditions are essentially the same as in autotransplantation of thyroid in the guinea-pig; there is a ring of preserved thyroid tissue surrounding a central necrotic zone. The latter is smaller in the rat than in the guinea-pig, because the transplanted lobe of thyroid is smaller in this case. Blood vessels and connective tissue cells are attracted by necrotic material in which is found the colloid of necrotic acini. The myxoid or edematous zone in the periphery of the organized center, which we observed in the guinea-pig, does not occur in the rat, although the center is here also well supplied with vessels and some loose connective tissue may occur in it. In the rat, the organized center is composed of wavy fibrillar connective tissue arranged in parallel bundles separated by clefts, which possibly represent lymph spaces. There are large capillaries here also and some of the original transplanted arteries which probably serve as channels for the new vessels. On the whole, the organization progresses rapidly and it is

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usually completed by the fifteenth day. Lymphocytes appear in small numbers in the central or in the peripheral connective tissue; they may also be found in some lymph vessels. The peripheral part of the parathyroid is also preserved, but its center is replaced by connective tissue.

The ring of thyroid tissue consists of acini which adjoin each other closely. Mitoses in acinus cells occur. In the first week after transplantation the colloid may be lacking or be incomplete in some acini; phagocytes may contribute to its destruction. Toward the end of this period the colloid has usually reassumed its solid character and it is retracted; the epithelium is usually lower than in the remnant of the original, not transplanted thyroid. On the whole, the number of fibroblasts which migrate between the acini toward the center of the transplant is very moderate, and the collection of lymphocytes which may occur is very small. Both are caused by conditions which are independent of the relationship between transplant and host; in particular, the stimulation of fibroblasts is caused by the necrotic material in the center of the thyroid. This or other non-specific factors here may also cause the slight increase in lymphocytes which is occasionally observed. Sometimes we can observe that the lymphocytes collect around some foreign bodies which are accidentally introduced into the subcutaneous pocket made for the transplantation.

A change of importance in the autotransplantation takes place usually around the fourth week, but occasionally somewhat later; at this time the thyroid ring becomes transformed into a curved structure of thyroid tissue which often has a serpentine form. Such an alteration is perhaps brought about through the opening of the ring of thyroid tissue and the extrusion of the central connective tissue into the neighboring tissue or perhaps through absorption of the central fibrous tissue as the result of the activity of fibroblasts. The connective tissue around the thyroid may be partly hyaline in character; this may possibly be due to the organization of colloid material in the necrotic center of the transplant. There is a good supply of capillaries around the acini; but only occasionally are they surrounded by strands of fibroblasts. The acini are well formed and show low epithelium. Toward the end of the fifth week, lymphocytes may be lacking or be present only in very small collections in the transplant.

Between the fortieth and sixtieth day, the transplant begins to

resemble the normal thyroid, but the epithelium is usually lower than in the remnants of the original gland from which the pieces were removed and a very small amount of fibrous tissue may be found between some of the acini. There are no lymphocytes present or only a few in the lymph vessels.

If we compare the autotransplantation of thyroid in the rat with that in the guinea-pig, we notice their essential similarity. In both cases the ingrowth of fibroblasts is very restricted and apparently is caused in the main by the presence of necrotic material in the center of the transplants. There is also the same tendency toward the formation of loose connective tissue in the center adjoining the thyroid ring, but this is more pronounced in the guinea-pig than in the rat. In both animals the central connective tissue is eliminated after some time; though how far an actual solution of this fibrous material takes place or what part is played by the pushing of the fibrous tissue to the outside of the transplant is uncertain. Furthermore, in both the guinea-pig and the rat the vessels are attracted by the thyroid transplant which gives off substances of an "auto" character and in both cases the lymphocytic collection which may be observed is very restricted and non-specific, caused by slight disturbances connected with the process of transplantation or by the accidental presence of foreign bodies. In autotransplantation of thyroid, therefore, in these animals the original injury to the thyroid tissue, with destruction of colloid in acini, is gradually repaired, and the transplant assumes more and more the character of the normal gland, although the epithelium of the acini becomes on the whole lower than in the autochthonous gland.

### B. Homoiotransplantation

We carried out three series of homoiotransplantation of the thyroid in the rat. In the first series donor and host had been obtained from different cities; we were therefore justified in our conclusion that they were not related to each other. In a second series of white rats host and donor were also usually not related to each other, but we could not in some cases exclude a distant relationship with the same certainty as in the first series. In a third series of transplantations, host and donor had different color patterns and had also been received from different cities. In describing our results, we shall ex-

tend the use of grades which we employed in a former paper dealing with homoiotransplantation of the thyroid in the guinea-pig. However, this grading can be used only at a period following transplantation when the reaction is fairly definitely developed, beginning with the eighteenth day. Tentatively it may also be used at somewhat earlier periods following transplantation. We had previously made use of such a system of grades in analyzing compensatory hypertrophy in the thyroid of the guinea-pig.

We shall first compare the results in Series I and II. Twenty-nine transplantations were used in the first series, and seventy-five transplantations in the second series. Thirteen transplantations constitute the third series. In the early period up to the eighth day following transplantation, about two or three rows of acini, or sometimes less, remain preserved. The acini are small and colloid has been lost in many of them, but it is preserved in others. Through the thyroid ring, especially through interstices, capillaries grow toward the center; some of them are quite large at this period. Mitoses may be seen in acinus cells. Toward the end of this period the necrotic center of the transplant is in some cases organized by not very dense small-celled connective tissue or sometimes by dense connective tissue; in other cases some necrotic material may still be left. It is taken up and removed, at least a part of it, by phagocytes. Hemorrhages may also occur from the newly formed capillaries which penetrate into the center. From the sixth day on, lymphocytes appear in some cases, at first in lymph vessels which penetrate through the thyroid ring; toward the end of this period they may begin to collect at the periphery of the center and may even fill the center diffusely; connective tissue as well as some lymphocytes also occasionally surround some acini. The peripheral part of the parathyroid may also be preserved, while the center is necrotic and in process of organization. Mitoses occur in the parathyroid as well as in thyroid cells. There is no noticeable difference between the two series at this time.

In Series I from ten to fifteen days after transplantation, the "homoio" character of the transplant is usually pronounced, but certain variations occur. Sometimes no thyroid tissue is found, but only fibrous tissue with lymphocytes. In most cases the lymphocytic infiltration is marked and in some instances so intense that the appearance of lymph glands is simulated. Surrounded by masses of

lymphocytes there are some isolated acini or bundles of acini still visible. In other transplants the lymphocytes penetrate between and into the acini and destroy them. The center is usually converted into fibrous tissue, the amount of fibrous tissue present being much greater and the fibrous character much more pronounced than in the case of autotransplantation. In the beginning of this period, the organization of the central necrotic material is not yet complete in every case. The blood vessels in the center are no longer very conspicuous in the second half of this period. The thyroid ring when present is incomplete. However, in one case the transplant was better preserved and the central connective tissue smaller in quantity, so that the piece approached in certain respects the structure of an autotransplant except that the lymphocytic infiltration was much more pronounced. There seems to be some indication that the homoio-reaction is especially marked after transplantation in certain strains of rats.

At sixteen and seventeen days, we find again some variation in the condition of the homoiotransplants of the first series, especially as far as the amount of preserved thyroid tissue is concerned. In some cases it is considerable but in other cases very slight. In the latter condition the colloid is lost in the majority of the remaining acini. Fibrous bands separate the acini or bundles of acini. Lymphocytes also penetrate into the thyroid tissue and separate acini in certain areas. The centers of the transplants as well as the lymph vessels are filled with lymphocytes.

In Series II, conditions are somewhat similar from the tenth to the twelfth day. However, in general the thyroid ring is somewhat better preserved in this series. At ten days the center is not yet completely organized in every case. Connective tissue and lymphocytes surround some acini. At twelve days there is noticeable for the first time a considerable lymphocytic infiltration. If we compare the transplants of the first and second series between the thirteenth and eighteenth day, we find in both a similar condition, but the average preservation is slightly better in Series II. In both the first and second groups the preservation at this time is better than at the later period, from twenty to thirty days. The latter period seems to represent the critical time in which the destruction has reached its maximum. At the thirteenth day, the center in Series II is usually organized by fibrous tissue but some loose con-

nective tissue is also occasionally found there. The colloid may be preserved or partly destroyed; in the peripheral acini especially it is often preserved. Fibrous bands surround the acini. There is moderate or marked lymphocytic infiltration in the center as well as in the periphery of the transplant and also between the acini. The parathyroid is likewise much infiltrated with lymphocytes. At fifteen days, there is found in one transplant a condition resembling that of a syngenesiotransplant: loose connective tissue, fat tissue and blood vessels are seen in the center; there is slight lymphocytic infiltration in the capsule of the thyroid and from here lymphocytes penetrate in places between the acini; also some lymphocytic infiltration is found in the center of the transplant; mitoses occur in thyroid as well as in parathyroid cells. But in other specimens at this period, lymphocytes and connective tissue have destroyed the greater part or the whole of the thyroid, and connective tissue surrounds and separates the remaining acini from each other. At seventeen days, there is usually much lymphocytic infiltration in the periphery of the transplant, mainly around the vessels. The fibrous center is large and wide vessels pass toward it through the thyroid ring. Lymph vessels are filled with lymphocytes and there is some indication that lymphocytes can migrate into the lumen of the vessel from the surrounding tissue. Lymphocytes also enter the parathyroid; however, the lymphocytic infiltration varies in amount in different cases. Occasionally mitoses are still found in acinus cells.

In order to obtain a quantitative measure of the strength of the homoio-reaction we arranged the various transplants in six classes as follows: Grade 6 corresponds to an autotransplant. Grade 5 is similar to a syngenesiotransplant; the structure of the transplant may resemble that of an autotransplant or the amount of connective tissue in the center may be slightly increased; but there is a definite lymphocytic infiltration noticeable. In grade 4 there is a well preserved thyroid ring, but there is an increase in fibrous tissue around the acini and the lymphocytic infiltration is quite marked in places. The center is fibrous and enlarged. In grade 3 there is still a thyroid ring present, but it is thin and may be not quite complete or there are at least larger bundles of acini preserved. The increase of fibrous tissue surrounding the acini is very pronounced and the lymphocytic infiltration is also marked. The center consists likewise of dense fibrous tissue which is present in a relatively considerable

quantity. In grade 2 only isolated acini or bundles of acini are found in the fibrous tissue. The lymphocytic infiltration is quite marked. In grade 1 the thyroid proper has been entirely destroyed and only fibrous tissue and some lymphocytes are found.

If we use this classification, we find in the period between the thirteenth and eighteenth day in Series I an average of grade 3 and in Series II of 3.4. That means that the preservation of the transplant is slightly better in Series II.

SERIES I. 19 TO 40 DAYS. In this period after transplantation there are in Series I thirteen experiments with an average grade of 2.5, while in Series II there are twenty-seven experiments with an average grade of 2.7. Between twenty and thirty days the maximum in the homoio-reaction is obtained; the destruction of the transplants is greatest.

The condition found in Series I is indicated by the following grades. *20 days:* (a) grade 1, much lymphocytic reaction. (*b*) grade 1. (*c*) grade 4, thyroid ring, in places dense lymphocytic infiltration; in other places the lymphocytic infiltration not dense; most acini without colloid, some with colloid. (*d*) grade 1, lymphocytes present. (*e*) grade 3 or 3–4; there are one or two rows of thyroid acini; acini separated by much fibrous tissue and much lymphocytic infiltration; colloid lacking in acini. (*f*) grade 2; there are in the fibrous tissue some bundles of compressed acini the lumina of which are small slits; intense lymphocytic infiltration. *21 days:* grade 3, thyroid ring, acini with solid colloid or compressed slits; fibrous tissue with much lymphocytic infiltration surrounding acini. *24 days:* grade 2. *25 days:* grade 1. *30 days:* (*a*) grade 2–3; in periphery remnants of large and small acini with colloid; very dense lymphocytic infiltration. (*b*) grade 1 or 1–2. *40 days:* (*a*) grade 1. (*b*) grade 1. (*c*) grade 5, syngenesio-reaction, auto-structure with some masses of lymphocytes. (*d*) grade 5. The number of the experiments at different periods is relatively small in this series, but there is some indication that at forty days in some cases a recovery may have taken place in the condition of the homoiotransplants.

SERIES II. 19 TO 40 DAYS. This impression becomes more definite if we compare in Series II the grades of preservation and lymphocytic and connective tissue reaction in the periods from nineteen to thirty days with those in the periods between thirty and forty days.

*At 19 days:* grades 2, 2–3, 2–3, 2–3, 3, 1, 1. There is as a rule only a small amount of thyroid tissue preserved. The lymphocytic and connective tissue reaction is usually pronounced; lymphocytes as well as connective tissue help to destroy acini which are quite commonly without colloid. Lymphocytes separate and penetrate actively into acini. However, in some cases the fibrous tissue reaction is more pronounced than the lymphocytic invasion. On the whole the parathyroid, after its central part has once been organized, is usually less invaded and injured by connective tissue and lymphocytes than the thyroid tissue, but lymphocytes and connective tissue may also enter the parathyroid and partly destroy it.

*At 20 and 21 days:* grades 5, 2, 1. In one case, only fibrous tissue with some lymphocytes is found (grade 1). In the second case (grade 2), the transplant appears almost like a lymph gland. The large majority of the acini are destroyed, but a number of acini are still preserved and some of these contain colloid. Lymphocytic masses penetrate into the remaining acini from the outside as well as from the inside of the thyroid ring and thus help to destroy them. In one case (grade 5), the structure of the transplant approaches that of an autotransplant; it has the form of an ellipsoid. There is a small amount of loose areolar connective tissue in the center. In addition to some diffuse lymphocytic infiltration there are in places small collections of lymphocytes around vessels. In this case we have to deal with a typical syngenesio-reaction.

*At 23 to 25 days:* grade 2; only some islands of acini left; some acini still with colloid; almost all of the thyroid has been replaced by lymphocytic masses. Grades 1, 3, 1, 1, 2–3; acini without colloid form small clefts in the fibrous tissue; they are individually surrounded by fibrous tissue. There is marked lymphocytic infiltration. Less than one-half of the periphery still contains some acini. The peripheral part of the parathyroid is preserved.

*At 30 days:* grades 1, 2, 3; some parts of thyroid ring left; some acini with, some without colloid. There is an intense diffuse lymphocytic infiltration. Lymphocytes penetrate between the acini; bundles of acini, lying close together, are surrounded by lymphocytes. The center is filled with lymphocytes. Grade 2–3, only a small part of thyroid preserved; still some acini lying close together, with or without colloid. The majority of the acini are compressed and are without colloid; they are surrounded by fibrous tissue. In

some places, a very marked lymphocytic infiltration; in other places only few lymphocytes enter, disorganize and destroy acini. The surrounding connective tissue is very fibrous. Grade 4, rather well preserved ring of acini; the majority of acini are without colloid, some are with collcid. Fairly marked lymphocytic infiltration. A few lymphocytes are in periphery; the majority are in the center. Some fibrous bands around acini.

*At 31 to 40 days:* grade 5; auto-structure: ellipsoid of thyroid, acini close together. A little fibrous tissue and a very extensive lymphocytic infiltration in center. In several places in periphery collection of lymphocytes. Some lymphocytes penetrate between acini at certain points. Well preserved parathyroid with a number of mitoses in parathyroid cells; beginning lymphocytic infiltration in periphery of parathyroid. Grade 6, about like autotransplant; acini close together with good retracted colloid; in center areolar and some fibrous tissue, and also some blood pigment. Grade 5, in this case especially around parathyroid marked lymphocytic infiltration. Grade 4–5, very large and well preserved ring of acini, but increased amount of fibrous tissue in center. There is a slight diffuse and more severe localized lymphocytic infiltration, especially around parathyroid. Lymphocytes penetrate also a little between acini, but there is no destruction of acini. Grade 5, only in places in center small collections of lymphocytes. Grade 3–4, a number of follicles with colloid, some compressed acini. Much fibrous tissue in center. Intense infiltration with lymphocytes; in periphery collection of lymphocytes. Some parts of thyroid almost completely replaced by connective tissue.

We see in this series a distinct improvement in the preservation of the transplants and a diminution in the intensity of the lymphocytic reaction between the thirtieth and fortieth day. This improvement is maintained between the fortieth and eighty-fifth day after transplantation.

*At 40 to 85 days*: These experiments were carried out with animals of Series II. In two animals after forty-two days only epidermal cysts are found; they are derived from the strands of stratified epithelium which are admixed to the thyroid and parathyroid glands in the guinea-pig as well as in the rat. The grades in the other cases were as follows: *42 days:* grade 5, well formed acini with colloid; little connective tissue, some blood pigment in center. In certain

places, especially at both poles of the transplant and between acini, some lymphocytic infiltration; grade 5. *47 days:* grade 5; both similar to first specimen; in latter specimen only small parts of thyroid destroyed by lymphocytes. *49 days:* grade 3.5, a considerable number of well preserved acini; some with, but the majority without colloid. Large fibrous center. Intense lymphocytic infiltration. Lymphocytes invade and destroy many acini; lymph vessels are filled with lymphocytes. Also epithelial cyst is infiltrated with lymphocytes. Considerable part of thyroid is destroyed. *50 days:* grade 5; auto-structure, but in various places in center and also in periphery of transplant collections of lymphocytes, especially around blood vessels; they penetrate also a little between acini. Lymph vessels in center and in periphery filled with lymphocytes. *54 days:* grade 4; fairly good thyroid ring; at both poles lymphocytic masses. Around vessels in center and between acini considerable lymphocytic infiltration. *85 days:* grade 5. This experiment, in which donor and host were obtained from different cities, does not strictly belong to this series. We find auto-structure, but in several places masses of lymphocytes in lymph vessels and in connective tissue are seen and they begin to invade the thyroid. Grade 5.5; auto-structure; good colloid in acini. Around one lymph vessel in center collection of lymphocytes. The average grade of this series is 4.82.

We see thus, after the maximum of destruction has been reached in the course of the fourth week following transplantation, a rise in preservation of thyroid and a diminution of destruction of thyroid by lymphocytes. In evaluating these experiments we must consider that the experiments with the longest duration were carried out with animals of Series II, in which a relationship between donor and host could not be excluded with the same certainty as in Series I. It is therefore possible that a closer relationship between the animals may in part explain the result. On the other hand certain facts suggest the possibility of a gradual adaptation occurring between the transplanted tissue and tissues of the host. How far the one factor and how far the other two factors come into play, has to be investigated in further experiments, and these are being carried out at the present time.

SERIES III. In this series we transplanted the thyroid from white rats into two kinds of pure colored strains, a cream and a hooded strain which had been bred by Dr. Helen D. King, and which we

received through her kindness from the Wistar Institute. One transplant was examined after seven days, all the others were examined after twenty and twenty-one days.

*After 7 days*: From HaO white rat to a cream rat. There is a marked homoio-reaction. The acini are mostly without colloid, but some are with colloid. There is much connective tissue growing between the acini and consequently many acini are compressed and have a very small lumen; there is also a distinct connective tissue capsule around the transplant with definite lymphocytic infiltration. Collections of lymphocytes are found in lymph vessels which together with connective tissue enter the transplant.

All the other pieces were examined *twenty and twenty-one days* after transplantation. I. From HaO white rats into cream rats. (*a*) grade 1. (*b*) grade 1–2; fibrous tissue nodule with moderate lymphocytic infiltration; a few acini without colloid left, but in process of destruction; the large majority of the acini have been destroyed. Moderate lymphocytic infiltration in connective tissue; some lymphocytes invade acini. Connective tissue is still in process of organizing necrotic parts of the transplant. (*c*) grade 1. (*d*) grade 1–2; loose connective tissue with blood pigment in the center. Marked lymphocytic infiltration around some remnants of compressed acini which are being invaded and destroyed by lymphocytes. (*e*) grade 1. (*f*) grade 1; fibrous tissue with dense lymphocytic infiltration.

II. From white Micha rats to hooded rats. (*a*) grade 1. (*b*) grade 1. (*c*) grade 1; fibrous tissue and lymphocytic infiltration. (*d*) grade 1. (*e*) grade 1; dense lymphocytic infiltration. (*f*) grade 1.

As early as seven days after transplantation there is a very marked homoio-reaction noticeable in this series. There is much fibrous tissue formation around the transplant and between the acini. The acini are compressed and there is a distinct lymphocytic infiltration. As a result of these processes which evidently continue to act during the second and third week, we find twenty to twenty-one days after transplantation in ten cases a complete destruction of thyroid tissue (grade 1), while in only two cases small remnants of thyroid are left; these are very seriously injured as a result of the action of connective tissue and lymphocytes and they are near the point of complete destruction (grade 1–2). The reaction of the host against the transplant is therefore decidedly more marked and successful in case of exchange of organs between white and cream or

hooded rats than in cases in which organs were exchanged between non-related white rats. There is no essential difference between the action of cream and of hooded rats on thyroid transplants of white rats. The reaction is possibly slightly more marked in the case of the hooded rats.

### Homoiotransplantation of Uterus and Ovaries in White Rats

In five animals, pieces of uterus, with or without ovaries, were homoiotransplanted into animals in which also the thyroid from the donor had been transplanted. The examination was carried out fifteen to twenty-three days after transplantation. In the thyroid transplant there has been in each case a very marked homoio-reaction; either the lymphocytic reaction has been very pronounced or the thyroid has been entirely destroyed. The transplanted uterus has also been partly destroyed; there is either lymphocytic infiltration in the transplanted muscle tissue and epithelium or at least in the adjoining fat tissue.

### Summary

1. In all essential respects the laws governing autotransplantation of thyroid and parathyroid in the rat are the same as those established in the case of the guinea-pig. Connective tissue seems in both cases to be attracted by necrotic material; slight collections of lymphocytes are due to non-specific conditions, such as accidental presence of foreign bodies. In both cases, a gradual elimination of these abnormalities takes place and the autotransplant more and more resembles the autochthonous organ. Some differences exist between the methods of regulation which serve to eliminate the adverse conditions in the rat and guinea-pig, but these differences are due to factors of secondary importance, such as the size of the transplant.

2. After homoiotransplantation of the thyroid and parathyroid, the same agencies of attack on the part of the host come into play in the rat as in the guinea-pig; furthermore the intensity of this reaction seems to be approximately the same in the two animals.

3. The intensity of the aggressive reaction depends upon the relationship of host and donor. We carried out three series of trans-

plantations in which the grade of relationship varied. In the third series in which we transplanted tissues from white rats to pure strains of hooded and cream rats respectively, the intensity of the homoio-reaction was greatest. There was a slight difference only between the two other series in both of which white rats served both as donors and hosts. The reaction was here slightly more intense in the first series, where a distant relationship between donor and host could be excluded with greater certainty than in the second series.

4. In our second series in which a remote relationship between donor and host could not be excluded with the same certainty as in the other series, a maximum of destruction was observed during the fourth week after transplantation. From then on, an improvement in the condition of the transplant was observed. How far accidental relationship determines this result, how far a gradual adaptation between host and transplant is responsible for it, have to be determined in further experiments.

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# AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF CARTILAGE AND BONE IN THE RAT*

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In the preceding paper we have studied the auto- and homoiotransplantation of thyroid and parathyroid in the rat and compared the results with those obtained previously in the guinea-pig. We used three different groups of rats in Series I, II and III and in particular we tested the effect of a still more distant relationship between host and donor by transplanting the thyroid from white rats to pure strains of colored rats. In this communication we shall discuss the fate of cartilage of three different groups of rats. In the majority of cases we transplanted thyroid and cartilage into the same individual. We are thus in a position to compare in an exact manner the action of homoio-toxins of various strengths on the cartilage with that on the thyroid. We furthermore made some observations on normal xiphoid cartilage and on cartilage after autotransplantation in the rat.

The normal, non-transplanted xiphoid cartilage in the rat is surrounded by fat tissue in which the connective tissue septa are fine. At a certain level, muscle tissue attaches itself to the perichondrium. The width of the cartilage varies in different places. It is thin towards the free margin and is thicker higher up. The thin tissue consists of vacuolar cartilage cells. When the cartilage higher up becomes thicker, the center of the cartilage often shrinks and becomes necrotic and connective tissue grows into it, replacing the necrotic part. Furthermore, when the cartilage becomes necrotic, the perichondrium at the outside begins to proliferate and to form a plate of new cartilage cells, which later produce much hyaline tissue; then again some solution processes may take place.

The conditions which lead to a necrosis of the transplanted normal cartilage (*i.e.*, lack of foodstuffs and oxygen) have the same effect in the non-transplanted normal cartilage. Furthermore, the perichondrium in this instance also produces plates of new cartilage

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alongside the necrotic cartilage. Connective tissue grows into the necrotic areas under normal conditions, just as in the case of transplantation. There is a structural difference between the xiphoid cartilage of the rat and of the guinea-pig, in the former the tendency to vacuolization on the part of the cartilage cells being greater than in the latter.

### Autotransplantation of Cartilage

A complete series of autotransplantations of cartilage was not undertaken in the rat, but pieces were examined between the seventeenth and twenty-sixth day after transplantation. On the whole, the cartilage is found in a normal condition, surrounded by perichondrium and fat tissue. The perichondrium is always free from lymphocytes. The fat tissue is either quite normal or occasionally it shows some necrotic areas. In some cases it is free from infiltration, but in other cases there are very slight collections of lymphocytes around vessels. The presence of the lymphocytes is not due to specific homoio-toxins, but is probably caused by necrotic changes and other accidental alterations in the fat tissue. Some small necrotic areas are also quite commonly found in the cartilage either at the end, or in the central parts of the transplanted tissue, where it is thicker and where there is some scarcity of food or oxygen. In certain cases the necrotic central part of the cartilage becomes dissolved. Buds of connective tissue invade and replace the necrotic areas. Occasionally necrotic pieces of cartilage are surrounded by connective tissue which has grown into the transplant. Furthermore, alongside areas of dead cartilage perichondrium produces, as usual, new plates of perichondrial cartilage. We see then that the autotransplant shows reactions similar to those observed in the normal non-transplanted cartilage.

### Homoiotransplantation of Cartilage

We shall discuss successively Series I, II and III of transplantations. In order to summarize our results, we shall adopt the same system of grades which we used in the case of the thyroid, and in each animal we shall compare the grades of the transplant with those of the corresponding thyroid transplant, obtained from the same animals. As we noticed previously in the guinea-pig, so also in the

rat the homoio-reaction is less marked in cartilage than in thyroid transplantations. Thus the differences between different homoio-transplants are less distinct in the case of cartilage and the grading is made somewhat more difficult. Furthermore, when cartilage is used, the transplants are found better preserved than the corresponding transplants of thyroid and the grades of reaction are therefore higher than with the latter tissue. Notwithstanding this difference between thyroid and cartilage, there will be apparent a correspondence between the results obtained in both tissues when transplanted into the same animal. We shall give first a summary of the results obtained in each series and compare in each case the grades of reaction against cartilage and thyroid and then cite a few examples to indicate the changes taking place in the cartilage transplants at various periods.

### Series I. White rats, not related

A. *From 20 to 25 days. 20 days.* (*a*) Only little of the surrounding fat tissue left. In places marked, in other places moderate, but always a distinct mantle of lymphocytes around cartilage; some penetration of lymphocytes into perichondrium. Around necrotic cartilage regenerated perichondrial cartilage plate. Grade 2 (thyroid grade 1). (*b*) Marked fibrous substitution of fat and areolar tissue; some fat tissue left; marked lymphocytic infiltration around cartilage and in fibrous tissue. Grade 1.5 (thyroid grade 1). (*c*) Cartilage partly living, partly necrotic. Fat tissue around cartilage; slight lymphocytic connective tissue thickening in fat tissue. No lymphocytes. Grade 4 (thyroid grade 4). (*d*) Much necrotic cartilage, regenerated perichondrial cartilage plate; fat tissue with fibrous nodes; few lymphocytes. Grade 3 (thyroid grade 1). (*e*) Necrotic center of thick cartilage invaded by connective tissue. Thickening of perichondrial tissue; new formation of perichondrial cartilage. Considerable areolar tissue left, but much of it invaded and replaced by fibroblasts; very densely infiltrated where fibroblasts proliferate. Grade 2.5 (thyroid grade 3 or 3.5). (*f*) Areolar fat tissue around cartilage, but also some dense fibrous tissue. In fibrous tissue, especially around remnant of fat tissue, here and there lymphocytic infiltration; mantle of lymphocytes around cartilage. Bone and proliferative zone of cartilage adjoining bone, preserved. Osteoblasts and osteoclastic giant cells. Grade 3 (thyroid grade 2).

*21 days.* Good cartilage. Fat tissue partly invaded and surrounded by connective tissue; much lymphocytic infiltration in connective tissue of fat. Bone partly preserved with osteoblasts. Much lymphocytic infiltration around muscle tissue. Grade 3 (thyroid grade 3).

*24 days.* Necrotic center of thick cartilage; perichondrial regenerated plate. Connective tissue growing into necrotic cartilage. Moderate connective tissue thickening in fat tissue. Grade 3.5 (thyroid grade 2).

*25 days.* Cartilage partly necrotic; regenerated perichondrial plate. Medium fibrous thickening of areolar tissue; lymphocytic infiltration around cartilage and fat tissue. Grade 3 (thyroid grade 1).

B. *From 30 to 40 days. 30 days.* (*a*) Good cartilage surrounded by areolar tissue. Some increase in connective tissue. In areolar tissue a number of strands of lymphocytes; also around cartilage some clumps of lymphocytes. Grade 3 or 3.5 (thyroid grade 2.5). (*b*) Necrotic areas are seen in thick cartilage; regenerated perichondrial cartilage plate. Increased connective tissue in areolar tissue and around cartilage. Fairly marked lymphocytic infiltration around cartilage or along perichondrium; no general infiltration. Grade 3 (thyroid grade 1 or 1.5).

*40 days.* (*a*) Good cartilage; small necrotic areas. Fibrous, fibrillar connective tissue, which encloses fat cells, around cartilage. Marked lymphocytic infiltration, extending also into periphery of living cartilage. Grade 1.5 (thyroid grade 1). (*b*) Good cartilage; mostly moderate lymphocytic infiltration. Grade 3.5 (thyroid grade 1). (*c*) Good cartilage surrounded by lymphocytes, areolar and fat tissue. Very moderate increase of lymphocytes. Grade 5 (thyroid grade 5). (*d*) Good cartilage, areolar tissue and fat tissue. Some necrosis with perichondrial cartilage regenerated. Very slight lymphocytic infiltration. Grade 5 (thyroid grade 5).

C. *Earlier transplants from 5 to 8 days. 5 days.* Cartilage partly living, surrounded by fat tissue; no connective tissue; no lymphocytic reaction. *8 days.* Cartilage partly living; some perichondrial newformation of cartilage. Good fat tissue and some increase of connective tissue around cartilage. Little nodes of connective tissue in fat tissue, no lymphocytes; chains of muscle nuclei; grade 2.5.

D. *From 10 to 12 days. 10 days.* (*a*) Good cartilage surrounded by fat tissue; some necrotic cartilage; slight connective tissue increase in fat tissue and around cartilage. No lymphocytic infiltration; perichondrial plate. Grade 3 or 4 (thyroid grade 2.5). (*b*) Good cartilage with necrotic areas in center; thickened fibrous tissue; areolar tissue with strands of connective tissue; much connective tissue proliferation; lymphocytic infiltration. Grade 2 or 3 (thyroid grade 2 or 3). *12 days.* (*a*) Good cartilage; some necrotic cartilage, perichondrial plate. Much increase in connective tissue around cartilage and fat tissue. Some epithelioid cells. Some fat still well preserved. Much lymphocytic infiltration. Muscle nuclear chains. Grade 2 (thyroid grade 1). (*b*) Slight increase of connective tissue in fat tissue. Some lymphocytes. Grade 3.5 (thyroid grade 3.5). (*c*) Good cartilage, areolar and fat tissue with strands of connective tissue. Much lymphocytic infiltration affecting, almost exclusively, areolar and fat tissue. Grade 2 (thyroid grade 2). (*d*) Lymphocytic reaction around cartilage in areolar tissue. Small areas of lymphocytes and slight increase of connective tissue in areolar tissue. Grade 2 or 2.5 (thyroid grade 3).

E. *From 13 to 17 days. 14 days.* Much necrotic cartilage, with substitution by perichondrial cartilage, surrounded by areolar and fat tissue with connective tissue strands. Moderately diffuse lymphocytic infiltration, more marked in areolar tissue than around cartilage. Grade 3.5 (thyroid grade 4). *15 days.* Good cartilage; where it is thicker, necrotic areas in center and a little perichondrial cartilage. Areolar tissue mainly fibrous; only remnants of areolar tissue; marked lymphocyctic infiltration in connective tissue of areolar tissue and around cartilage. Grade 1 (thyroid grade 1). *16 days.* (*a*) Much necrotic cartilage, with some perichondrial regeneration. Good fat tissue around cartilage; no connective tissue nor lymphocytic infiltration. Some connective tissue near transplanted muscle tissue and lymphocytes near point where cartilage forms bone. Grade 4.5 (thyroid grade 4). (*b*) Good cartilage; no connective tissue increase. Intense lymphocytic infiltration around cartilage and in fat and muscle tissue. Grade 4. *17 days.* Good cartilage. Some necrotic areas and perichondrial plates. In areolar tissue, nodular connective tissue increase and increase of connective tissue especially around vessels. Moderate lymphocytic infiltration. Bone mostly necrotic. Grade 3 (thyroid grade 2).

In this series the bone adjoining the cartilage was transplanted in some cases. We cite a few of the protocols of cartilage and also of bone transplantation.

*After 12 days.* (*a*) Chi = Ad. No thyroid found; the homoio-reaction in the cartilage transplant is quite pronounced. The cartilage itself is well preserved, except for an area of necrotic cartilage around which perichondrium produces a new cartilage plate. There is some increase in connective tissue in the fat tissue and around the cartilage, and epithelioid cells have formed in the fat tissue. In most places fat cells are separated by newly formed connective tissue, but some fat tissue is still unaltered. In the transplanted muscle there are either single nuclei or chains of nuclei, some of which are very large. Connective tissue separates the muscle fibers. There is much lymphocytic reaction around the muscle tissue. There is also much diffuse, though not very intense, lymphocytic infiltration in fat tissue, and some lymph nodes are filled with lymphocytes. (*b*) Ad = Po. The thyroid transplant has almost the character of an autotransplant, except for beginning lymphocytic infiltration. The cartilage and fat tissue also resemble auto-tissue in structure; but there is, in the fat tissue, in places an increase in fibroblasts with some lymphocytes. In the capillaries many lymphocytes are occasionally found. Cartilage and thyroid transplants correspond thus to each other.

*After 16 days.* Ad = Micha rat. There is considerable necrosis in the cartilage; especially where the cartilage is thicker, necrotic areas develop and cavity formation results. Around the necrotic cartilage the perichondrium produces new cartilage in places. Fat tissue around cartilage well preserved, and without any connective tissue increase or lymphocytic infiltration. There is also some transplanted muscle tissue seen and in this area connective tissue proliferation has taken place; some lymphocytic infiltration near cartilage. This piece has almost the character of an autotransplant, although the corresponding thyroid transplant shows a mild homoio-character. Toward the adjoining bone a proliferation of the perichondrial cells takes place and the cells enlarge without vacuoles forming; their cytoplasm stains blue with hematoxylin; intercellular substance is produced and bone formation results.

*After 17 days.* Micha = St. L. Bone is mostly necrotic, but in some parts bone corpuscles are still present.

*After 20 days.* From Chi to Ada rat. Cartilage is well preserved. There is mostly areolar connective tissue and fat tissue around cartilage, but also some dense fibrous tissue. In the fibrous tissue some lymphocytic infiltration is noticeable, especially around remnants of fat tissue; otherwise only here and there lymphocytic infiltration in the areolar tissue. Lymph vessels are filled with lymphocytes and a mantle of lymphocytes surrounds the cartilage. In the bone, bone cells are preserved and columns of cartilage cells are seen in the zone where cartilage becomes transformed into bone; there are also large capillaries here. Around the bone there are connective tissue and cells resembling osteoblasts and osteoclastic giant cells. In this case the homoio-reaction in the cartilage is less severe than in the thyroid.

*After 21 days.* From young Chi to old Ada rat. Cartilage is well preserved and partly surrounded by fat tissue in which there is considerable lymphocytic infiltration; some lymph vessels here are filled with lymphocytes. Connective tissue is growing into the fat tissue and the latter is thus gradually more and more invaded by it, the fat cells being surrounded by and included in the connective tissue. There is considerable lymphocytic infiltration in the connective tissue and much also in the transplanted muscle tissue. Within the lymphocytic masses there seem to be some small myoblasts. The bone is partly preserved, with osteoblasts surrounding it; in other places these cells are lacking. Osteoclasts also are found around the bone. In both thyroid and cartilage transplants, we find marked lymphocytic infiltration and in both the connective tissue is increased; there is much lymphocytic infiltration, especially around the muscle tissue.

We find thus, in this series, bone preserved in the homoiotransplants. In the zone of bone formation from cartilage there may be columns of cartilage cells which produce the material from which bone develops.

## Conclusions (Series I)

1. In all essential respects the homoio-reaction against cartilage in the rat is similar on the one hand to the homoio-reaction against thyroid in the rat and in the guinea-pig, and on the other hand to the homoio-reaction against cartilage in the guinea-pig, but there are some quantitative differences. (*a*) In the homoio-reaction

against cartilage in the rat, the lymphocytic and connective tissue reaction is less intense than in the homoio-reaction against thyroid in the rat or guinea-pig. On account of this weaker reaction the differences in the intensity of the homoio-reaction against cartilage in the rat are not so distinct as in the case of the thyroid and therefore the grading is not so easily applied. For this reason it is not possible to establish perfect parallelism between the individual grades of homoio-reaction against cartilage and thyroid in the rat; but in a general way a parallelism can be demonstrated. (*b*) If we compare the homoio-reaction against cartilage in the rat with that in the guinea-pig, we find that in general the invasion of fat and areolar tissue and its substitution by connective tissue is less pronounced in the former than in the latter animal. (*c*) The cartilage withstands more successfully than the thyroid the attack of lymphocytes and connective tissue.

2. In those rats in which the grades of homoio-reaction against thyroid varied between 1 and 2, 3 and 5, and 4 and 5, the corresponding average grades in the case of cartilage were 2.5, 3.84 and 4.3. There is thus a general correspondence between the grades of homoio-reaction against cartilage and thyroid. The homoio-differential against the cartilage and the thyroid of a certain individual is essentially the same on the part of the same host, but secondary factors may cause some minor differences.

3. It is possible to some extent to separate, in individual cases, lymphocytic from connective tissue reaction. Lymphocytic reaction may, at a certain time, be noticeable when the connective tissue reaction is weak, and conversely the connective tissue reaction may be marked when the lymphocytic reaction is almost lacking; but in general there is a parallelism between these two kinds of homoio-reactions in the case of rat cartilage as well as of rat thyroid transplantation.

4. The homoio-reaction against cartilage is usually quite distinct on the tenth day after transplantation, but it may begin a few days earlier. It reaches its greatest intensity in the fourth week, but there is a definite indication that at a still later date the intensity decreases again. This fact will come out more clearly in Series II, in which the observation extended over a longer period of time.

## Series II (1 to 85 days)

In this series the rats had in some cases been obtained from the same breeder. Fifty-two pieces of cartilage were examined microscopically. They were taken out at intervals varying between one and eighty-five days following transplantation.

A few abstracts from protocols may be cited to illustrate the changes which take place in this series at different periods after transplantation.

*14 days.* Cartilage transplant, grade 3 (thyroid grade 1). Where cartilage is thin, it is well preserved; where it is thick, there is central necrosis. Some of the thick cartilage is dissolved. Near the thick part, in places perichondrium also necrotic. Where cartilage is necrotic and perichondrium preserved, perichondrium proliferates slightly. At one point perichondrium is detached from thick cartilage and forms a plate of perichondrial cartilage, which runs parallel to transplant; it is still adherent to old cartilage at one end. In the center thick cartilage becomes soft, partly dissolved and forms apparently a myxoid tissue. At last cells and fibrils disappear. The surrounding fat tissue is markedly infiltrated with fibroblastic and fibrillar connective tissue which includes fat cells; considerable area of fat tissue still preserved. Some lymphocytes in this connective tissue, but not large masses. Some lymph vessels fairly well filled with lymphocytes.

*16 days.* Grade 3.5 (thyroid grade 2.5). Cartilage on the whole well preserved. Where it is thicker, center is necrotic. In some places where cartilage is thick, the perichondrial cartilage also is necrotic. Connective tissue grows in and sequesters necrotic piece. Cartilage surrounded on one side by fibrillar fibrous connective tissue, on the other side by fat tissue. Around cartilage some fibroblasts produce fibrillar tissue. Through solution, cavities develop in fat tissue. Thus cells become free and produce apparently a loose connective tissue. Distinct lymphocytic infiltration in places around cartilage. Around necrotic cartilage there is perichondrial newformation. Proliferating perichondrial cells produce cartilage tissue in necrotic center.

*19 days.* Grade 3.5 (thyroid grade 2.5). Where cartilage is thin, it is preserved; where it is thicker, it is necrotic. Around the latter places there is slight perichondrial cartilage formation. Some con-

nective tissue grows into necrotic hyaline cartilage and organizes it. Perichondrium, detached from cartilage near necrotic thick end, forms new plate of cartilage. Fat tissue surrounds cartilage, but some thickened connective tissue septa emanate from vessels, and here is also some lymphocytic infiltration.

*20 days.* Grade 3 (thyroid grade 3). Good cartilage surrounded by areolar tissue. Dense lymphocytic infiltration around cartilage and in areolar tissue. A few lymphocytes penetrate margin of cartilage; newformation of cartilage. Thyroid resembles in this case a lymph gland. Correspondingly there is marked lymphocytic infiltration in cartilage transplant; but absolutely the infiltration is less than in thyroid.

*20 days.* Grade 5 (thyroid grade 5). Good cartilage; central necrosis with perichondrial plate. Good areolar tissue; no new connective tissue; no lymphocytic infiltration around cartilage.

*25 days.* Grade 3 (thyroid grade 3). Vacuolar cartilage cells. Perichondrial regeneration. Newformation of connective tissue. Marked lymphocytic infiltration in fat and connective tissue.

*30 days.* Grade 3 (thyroid grade 2). Much perichondrial regeneration. Some perichondrial cartilage proliferation into necrotic cartilage. Well preserved fat and connective tissue around cartilage. Distinct lymphocytic infiltration in fat and connective tissue, but much less than in thyroid.

*40 days.* Grade 4.5 (thyroid grade 4.5). Good cartilage; some slight changes observed. The cartilage transplant is more like an autotransplant than is the thyroid. Where there is necrosis, some newformation of perichondrial cartilage has grown into necrotic area. In areolar and fat tissue some lymphocytic strands. Fibroblasts produce, in places, slight newformation of fibrous tissue and thus we find here and there some fibrous thickening.

*50 days.* Grade 6 (thyroid grade 5). Cartilage shows auto-reaction. No increase in fibrous tissue; no lymphocytic infiltration. In the thyroid transplant we find on the whole an auto-structure, but there are, in various places in the center as well as in the periphery, collections of lymphocytes, especially around blood vessels. They penetrate also slightly between acini. Lymph vessels in center and in periphery are filled with lymphocytes. Again the thyroid is a more sensitive indicator for homoio-toxins than the cartilage.

*85 days.* Grade 6 (thyroid grade 5.5). In thick cartilage, there

are necrotic areas and solution in center. The necrotic areas reach to connective tissue in places and the latter grows in and replaces necrotic material. At other side of cartilage much perichondrial cartilage regenerated, which may take the place of necrotic cartilage. The newly formed cartilage has at first small vacuoles which gradually enlarge. Even where cartilage is thinner, there are necrotic spots, substituted in part by regenerated perichondrium which forms cartilage. Perichondrium is thickened, and more so around thick than around thin cartilage. There is a large amount of good fat tissue around the transplant; no connective tissue thickening; no lymphocytic infiltration. Cartilage transplant corresponds to an autotransplant.

### Conclusions (Series II, 14 to 85 days)

1. In this series again a certain correspondence between the intensity of the homoio-reaction against the thyroid and the cartilage transplants is noticeable. In those animals in which the grades of reaction against the transplanted thyroid vary between 1 and 2, 2.5 and 4, and 5 and 6, the average grades against the cartilage transplant are respectively 3.1, 3.5 and 5.5. Furthermore, it is again apparent that the lymphocytic and connective tissue reactions are more marked in the thyroid than in the cartilage transplant. This difference is especially noticeable in those animals in which there is a marked homoio-reaction; in the animals in which the homoio-reaction is weak or lacking altogether in the case of the thyroid, it is also weak or lacking in the case of the cartilage. The destruction of tissue by lymphocytes and connective tissue is, on the whole, much greater in the thyroid transplant than in the cartilage. Moreover we are apt to find, in cases in which the thyroid transplant is so markedly invaded by lymphocytes that it almost resembles a lymph gland, that the cartilage transplant also is severely infiltrated. If we compare the degree of the homoio-reaction against cartilage in Series I and II, we find it somewhat more marked in Series I in which a relationship between donor and host can be excluded with greater certainty than in Series II. During the period between the nineteenth and twenty-sixth day, we find in Series I an average grade of 2.8, and in Series II an average grade of 3.3. We find again in Series II that the severity of the homoio-reaction gradually decreases and that in the course of time a recovery takes place in the

cartilage transplant. Thus between the nineteenth and twenty-sixth day the average grade is 3.3, while between the thirtieth and eighty-fifth day it is about 5.

2. The necrotic parts of the cartilage, especially those in the center of the transplanted tissue, can be replaced either by new cartilage produced through the activity of the adjoining perichondrium or by connective tissue which invades the necrotic areas. Which of these two processes occurs depends upon the state of preservation and reactivity of the perichondrium. It seems that necrosis of adjoining cartilage acts as a stimulus upon the perichondrium, which induces it to proliferate. In addition the perichondrium may proliferate alongside the necrotic cartilage and form a plate of new cartilage without actually replacing the dead parts. The new plates may be separated from the necrotic cartilage by a layer of connective tissue.

3. In several cases we observed that, through solution of cartilage, cartilage cells become freed from the surrounding intercellular substance and assume the appearance of myxoid connective tissue.

4. The recovery which we observed in the cartilage transplants at about the fortieth and fiftieth day following transplantation, is evident in the decrease in the intensity of the lymphocytic infiltration and in the quiescent state in which we find the connective tissue. Thus in several cases at later stages the transplant resembles autotransplants. Similar results were obtained in subsequent experiments with cartilage transplantation, and while it is possible that accidental variations in genetic relationship between donor and host may have played some part in bringing about the excellent condition in some of the transplants at later periods, there can be little doubt that a gradual diminution in the severity of the reaction against the strange tissue, on the part of the host tissue, does actually take place. An adaptation, perhaps akin to active immunity, may possibly produce this change in reaction.

We still have to consider the changes which take place in the cartilage and surrounding tissue in the first ten days after transplantation.

1. *In the first two days*, we find the cartilage itself well preserved, except where as a result of injury some necrosis occurred. Where the cartilage is thick, the center is necrotic and in the periphery a perichondrial cartilage plate has been produced. This took place

before transplantation. The fat and areolar tissue around the cartilage is on the whole preserved, but from the outside polynuclear leucocytes and some lymphocytes penetrate it. These cells may also enter into the cut end of the cartilage, when the cut has caused some necrosis; they furthermore invade necrotic cartilage elsewhere, as well as necrotic muscle tissue. The polynuclear leucocytes vary in number in different specimens, but on the whole they are not very numerous. They first surround the transplanted fat tissue and from here penetrate toward the center of the transplant.

*3 days.* Transplanted cartilage and fat tissue are well preserved. A few polynuclear leucocytes are seen here and there; also a few scattered lymphocytes around blood vessels. There is a slight connective tissue proliferation in the periphery around the fat tissue.

*4 days.* Polynuclear leucocytes have disappeared; lymphocytes are not observed. No connective tissue proliferation noticeable, except where connective tissue grows into necrotic cartilage. The greater part of cartilage preserved, but some central parts are necrotic and here solution processes have taken place. In the periphery, perichondrial cartilage had proliferated and produced new cartilage before the transplantation. Only small parts of the transplanted fat are necrotic.

*6 and 7 days.* Condition is similar, except that some proliferation of connective tissue is taking place and some lymphocytes and large mononuclear cells are seen around vessels; also in lymph vessels some lymphocytes and a few polynuclear leucocytes are seen. Here we have the first evidence of the homoio-reaction. This homoio-reaction is a little more distinct after seven days; lymphocytes and fibroblasts are seen in the interstices between the fat cells. Some septa in the fat tissue are thickened by lymphocytes and large mononuclear cells.

*8 days.* The fibroblasts have produced some connective tissue around the cartilage. Here, as well as in the corresponding thyroid transplant, lymphocytes are lacking. In a transplant of Series I at this period we find, at various points in the areolar or fat tissue, newly formed small nodes of connective tissue. In the muscle tissue some chains of nuclei can be observed. No lymphocytic infiltration; some perichondrial cartilage formation.

*9 and 10 days.* Cartilage mostly preserved surrounded by fat tissue, in which fibroblastic invasion has caused some connective

tissue thickening. In these connective tissue septa, are lymphocytes which penetrate also between fat cells. There may be in places already considerable substitution of fat tissue by connective tissue. Connective tissue with some lymphocytes is also growing into and replacing necrotic parts of the cartilage. Where cartilage is necrotic, there is either connective tissue proliferation or perichondrial cartilage regeneration, with plate formation already described. In various specimens there are variations as to the intensity of the lymphocytic and connective tissue reaction and they correspond to similar variations in the corresponding thyroid transplants.

### Conclusion (Series II, 1 to 10 days)

We see then that in the first three days there are slight collections of polynuclear leucocytes migrating into fat tissue in the direction toward the cartilage. They disappear after this period. Between the sixth and eighth day there begins a new formation of connective tissue and a slight infiltration with lymphocytes, which varies in strength in different specimens. There is a correspondence between the changes which take place in cartilage and in thyroid transplants at the same periods of time.

### Series III

This series includes transplantation of cartilage, of surrounding tissue, and in some cases of bone from white rats to cream and hooded rats. Simultaneously thyroid was transplanted into these animals.

1. *From white rats to cream rats. 7 days.* The greater part of cartilage necrotic; fat tissue partly necrotic. Connective tissue growing into living fat tissue. Connective tissue capsules around transplant. Bone necrotic. Moderate lymphocytic infiltration.

*20 to 21 days.* (*a*) Grade 2. Good cartilage; some newly formed fat tissue, with much fibrous tissue; moderate lymphocytic infiltration. Bone and zone of growing cartilage necrotic. (*b*) Grade 1.5. Limited amount of perichondrial cartilage. Much connective tissue; some fat tissue still left; dense lymphocytic infiltration. (*c*) Grade 1.5. Cartilage partly alive, partly necrotic. Around necrotic cartilage areas of newly formed perichondrial cartilage. Much connective tissue around cartilage and in fat tissue. Much lymphocytic infiltration. (*d*) Grade 2. Cartilage largely preserved, but some

necrotic areas. Fat tissue with increased connective tissue; medium lymphocytic infiltration. Bone necrotic, some fibroblasts invading it. Near bone a collection of what appear to be muscle cells, with large chains of nuclei. (*e*) Grade 2. Some bands of perichondrial cartilage. Much fibrous thickening in the fat tissue, but the lymphocytic infiltration here only moderate. Bone and bone-forming area of cartilage necrotic. (*f*) Grade 1.5. Cartilage partly alive, partly necrotic and surrounded by fibrous tissue, which has also replaced necrotic fat tissue. Preserved fat tissue with fibrillar thickening; moderate lymphocytic infiltration in some places, marked lymphocytic infiltration in others.

2. *From white rats to hooded rats.* (*g*) Grade 1.5. Fat and cartilage partly necrotic. Considerable increase in connective tissue; much lymphocytic infiltration. (*h*) Grade 1.5. This cartilage well preserved; where it is thicker, much necrosis in center. In periphery some newformation of cartilage; very rudimentary cartilage plate. Much connective tissue formation and lymphocytic infiltration. Bone and cartilage-growth zone necrotic. (*i*) Grade 1.5. Only parts of cartilage necrotic. Cartilage surrounded by fat tissue in which there is much connective tissue newformation. In places some lymphocytic infiltration in connective tissue around cartilage, but here, on the whole, it is moderate. Around bone, lymphocytic infiltration somewhat more marked. In bone-producing zone of cartilage, nuclei shrunken or chromatolytic and cartilage cells vacuolar. Bone entirely or almost entirely necrotic. (*j*) Grade 1.5. Cartilage vacuolar, partly necrotic, surrounded by fibrous tissue. Much fibrous tissue increase in and around fat tissue. In fibrous tissue around cartilage and in fat tissue, marked lymphocytic infiltration. Some epithelioid and giant cells in fat tissue. (*k*) Grade 1.5. On the whole good cartilage; only where it is thicker, a great part of it shrunken and necrotic. In surrounding tissue much increase of connective tissue, but some fat cells left. Dense lymphocytic infiltration around cartilage and in connective tissue, also in connective tissue around bone. Bone necrotic with some connective tissue growing into it. Cartilage-growth zone near bone is necrotic. (*l*) Grade 1.5. Good cartilage. Where it is thicker, some necrosis in center. Fat tissue partly replaced by fibrous tissue. Some coalesced fat cells. Dense lymphocytic infiltration in fat tissue and connective tissue around cartilage. Lymphocytes penetrating slightly into

perichondrial tissue; between fat cells marked lymphocytic infiltration.

A comparison of the grades in this series with the grades in the first series of transplants, both examined seventeen to twenty-five days after transplantation, is of interest. In the transplants from white to cream rats the average grade is 1.75; from white to hooded rats it is 1.5. The corresponding grades of transplants from white to non-related white rats is as follows: *17 days:* 2.75. *20 days:* 2, 1.5, 4, 3, 2.5, 3. *21 days:* 3. *24 days:* 3.5. *25 days:* 3. The average grade is 2.8. The transplants are therefore decidedly more injured after transplantation into cream and hooded than into white non-related rats. A similar result had been obtained in the thyroid transplants. Here the transplants were completely or almost completely destroyed in the cream and hooded rats, while in Series I the result was somewhat better. Furthermore, in the case of both thyroid and cartilage transplantations, the cream rats were slightly more favorable as hosts than the piebald rats. In this series also the destruction went further in the transplanted thyroid than in the transplanted cartilage.

## Conclusions (Series III)

An analysis of the processes which lead to these results shows the following: That as early as seven days after transplantation, there is much fat and cartilage tissue found to be necrotic, and some connective tissue growth into living fat tissue, as well as around the transplant, has taken place. The lymphocytic infiltration is moderate and less intense than in the case of the thyroid. After twenty to twenty-one days, the cartilage may be well preserved, except that there are found central necrosis and solution processes in places where the cartilage is thick. In a number of instances also other parts of the cartilage are found necrotic; but this is probably due to coincidence, because in other cases the cartilage is well preserved. In contradistinction to the cartilage, a complete destruction of the transplanted thyroid has taken place. Even a newformation of perichondrial cartilage can occur around necrotic areas of this tissue, but the regeneration is limited in amount; in some cases it only leads to production of abnormal structure resembling myxoid tissue. There is, therefore, noticeable on the part of the toxins an inhibiting influence on the regeneration of perichondrium. The transplanted

bone in this series is found completely or almost completely necrotic; also the proliferating zone of the cartilage adjoining the bone is invariably necrotic. The nuclei here may be shrunken or chromatolysis may occur and the cartilage cells are vacuolar. The toxic substances produced as a result of incompatability between transplant and host are injurious to the preservation of this tissue.

There is in this series always much newformation of fibrous tissue in the transplant, usually around the cartilage and always in the fat tissue; a great part of the latter may be replaced by fibrous tissue and only some parts of fat tissue are left. In the remaining fat, epithelioid and giant cells are found in some cases; also a coalescence of fat cells is occasionally observed. Connective tissue may grow into the necrotic cartilage, taking its place and penetrating in places into the bone. The lymphocytic infiltration is usually marked, but in some cases more moderate. These cells are found in the fibrous tissue around cartilage and in the fat tissue, around blood vessels and in lymph vessels. There is also noted lymphocytic infiltration around the bone. In one case remnants of muscle tissue with nuclear chains are found. On the whole, therefore, the reaction on the part of connective tissue and lymphocytes against the transplant is very marked. It is more intense than in Series I and II. In addition there is found in Series III complete necrosis of bone and of bone-forming cartilage. The regenerative activity on the part of the perichondrium is not entirely destroyed but decidedly inhibited.

### Homoiotransplantation of Bone Marrow

In the first series six transplantations of bone marrow were carried out, pieces being taken out for microscopic examination after 8, 12, 16, 17, 20 and 21 days. We found in every case a growth of connective tissue, usually accompanied by some lymphocytes, into the bone marrow, and thus a partial substitution of bone marrow by fibrillar connective tissue; only in one case was there bone marrow entirely replaced by fibrillar connective tissue.

If we compare these results with those obtained in the third series, we find in the latter that the destruction of bone marrow is more intense. The bone marrow is in every case completely necrotic and supplanted by fibrillar connective tissue, which in a number of cases is accompanied by lymphocytes. Some areas of solution are

also observed in the bone marrow. As early as seven days after transplantation in Series III the bone marrow is completely necrotic, and it does not have the power to recover from the injury received as a result of homoiotransplantation.

## Summary

1. Three series of experiments were carried out, in which cartilage was transplanted in three groups of rats which differed from each other in the degree of relationship between donor and host in each series.

A definite correspondence was established between the degree of genetic relationship between donor and host in each series and the severity of the reactions against the transplant on the part of the host tissues, in particular of the lymphocytic infiltration and the invasion and replacement of transplanted fat tissues by fibrous tissue.

This applies to the homoio-reactions of Series I and II and also of Series III. In the latter, where we have to deal with a further-going genetic difference between host and transplant, we find in addition a necrosis of bone and of the bone-producing zone of proliferating cartilage cells. Furthermore, while in Series I and II the regenerative function of the perichondrium is not interfered with, it is inhibited, although not entirely prevented, in Series III.

2. The difference between Series I and II on the one hand and Series III on the other hand comes out also in the fat tissue of transplanted bone marrow. In Series III it becomes entirely necrotic and is replaced by fibrous tissue at an early date, while in Series I and II part of it may remain active for some time. In general, bone marrow is a sensitive tissue which, under the influence of homoio-toxins, is readily injured after transplantation and does not at all or only very incompletely recover after homoiotransplantation. It thus seems to behave in a similar manner to unstriated muscle of uterus after transplantation.

3. Under conditions in which thyroid tissue is entirely destroyed by the reaction of the host tissue, cartilage remains alive to a large extent. If in these series lack of close genetic relationship causes any partial necrosis of the cartilage at all, it has this effect only in certain cases; even in Series III the cartilage may remain very well preserved.

4. In Series I and II the homoio-reaction against cartilage, instead of becoming more intense, on the contrary decreases in intensity in the course of time. An adaptation takes place apparently between transplant and host.

5. In autotransplantation cartilage and fat tissue behave in a manner similar to normal non-transplanted tissue. In places where the cartilage tissue is further removed from the sources of oxygen and food material, degeneration occurs in normal as well as in autotransplanted tissue and the perichondrium may regenerate and produce perichondrial cartilage.

# ADENOMAS OF THE ISLANDS OF LANGERHANS *

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Benign tumors of the pancreas are rare, particularly adenomas of the islands of Langerhans. I have been able to find only fifteen cases recorded in the literature. Yet in this paper I am able to report four cases, two from the Boston City Hospital. In addition, one already reported [1] was encountered in studying the material at the Boston City Hospital. Ordinarily but little attention is paid to the pancreas at necropsy, and a small adenoma may easily escape notice. In fact, their discovery is usually due to a chance inclusion in the section taken for microscopic examination. Thus the rarity of the lesion is probably only apparent.

It is difficult to lay down criteria for the diagnosis of this condition. Cecil [2] believes that the masses represent hypertrophied islands rather than true tumors. Certain of the cases reported as adenomas very likely should be interpreted as hypertrophic islands. Thus the large island 1.5 mm. in diameter reported by Ssobelew [3] in a diabetic is probably not a tumor. Similarly, Reitmann's case [4] and that of Herxheimer,[5] both associated with diabetes, hardly have the characteristics of a tumor. The case reported by Lang [6] presents a good deal of difficulty in classification, but can best be considered both hyperplasia and adenoma formation.

In determining the diagnosis of the four cases reported here, the following rules have been followed. The morphology and arrangement of the cells must resemble those of the islands. There must be a definite capsule, with compression of the adjacent pancreatic tissue. The mass must measure at least one millimeter in diameter.

That size alone does not count can be seen from the illustrations of Case III (Fig. 2) and of a large island from a non-diabetic pancreas (Fig. 3). The definite capsule and compression of the surrounding pancreatic tissue can be readily seen in the case of the adenoma, but the large island (Fig. 3) differs from a normal island in size alone.

* Received for publication May 13, 1926.

In the accompanying table the various cases are summarized.

| Case No. | Described by | Sex | Age in years | Diabetes | Cause of death | Size of adenoma |
|---|---|---|---|---|---|---|
| I | Warren | ♀ | 53 | — | Carcinoma of ovaries | 1.7 x 1.7 x 1.4 mm. |
| II | Warren | ♂ | 63 | — | Myocarditis | 1.3 x 1.1 mm. |
| III | Warren | ♀ | 49 | — | Pernicious anemia | 1.2 x 1.2 mm. |
| IV | Warren | ♀ | 48 | — | Chronic nephritis | 9.0 x 6.5 x 6.0 mm. |
| V | Nicholls [7] | ? | ? | ? | ? | 3.0 x 2.5 mm. |
| VI | Helmholz [8] | ♂ | 65 | — | Cardio-renal | 5.0 x 5.3 mm. |
| VII | Morse [1] | ♀ | 44 | — | Peritonitis | 5.0 x 5.0 mm. |
| VIII | Morse [1] | ♂ | 46 | — | Apoplexy | 3.0 x 3.0 mm. |
| IX | Alezais and Peyron [9] | ? | ? | ? | ? | 3.0 x 3.0 mm. (?) |
| X | Heiberg [10] | ♀ | 64 | + | Diabetic coma | 6.0 x 5.0 mm. |
| XI | Cecil [2] | ♂ | 63 | — | Carcinoma of colon | 4.0 x 3.5 mm. |
| XII | Rollett [11] | ♀ | 25 | — | Tuberculosis | 11.0 x 11.0 mm. |
| XIII | Lecomte [12] | ♀ | 42 | — | Tuberculosis | 100.0 x 70.0 x 40.0 mm. |
| XIV | Koch [13] | ♀ | 22 | — | Bronchopneumonia | 14.0 x 14.0 mm. |
| XV | Priesel [14] | ♀ | 63 | — | Lobar pneumonia | 12.0 x 10.0 x 9.0 mm. |
| XVI | Priesel [14] | ♂ | 59 | — | Bronchopneumonia | 10.0 x 9.0 mm. |
| XVII | Priesel [14] | ♂ | 60 | — | Arteriosclerosis | 35.0 x 25.0 x 10.0 mm. |
| XVIII | Schneider [15] | ♂ | 84 | — | Nephritis, bronchopneumonia | 15.0 x 10.0 mm. |
| XIX | Schneider [15] | ♀ | 70 | — | Tuberculosis | 50.0 x 45.0 x 30.0 mm. |
| XX | Lang [6] | ♀ | 39 | — | Postoperative bronchopneumonia | Up to 5 mm. |

CASE I. H–26–177. Female, 53 years old. Clinical diagnosis: Inoperable abdominal carcinoma. Anatomic diagnoses: Carcinoma of ovaries, with metastases to peritoneum and liver.

The pancreas is negative in gross. Microscopic examination shows a thin layer of atypical epithelial cells along the anterior surface, a metastasis from the ovarian carcinoma. On one of the slides, of tissue from the body of the pancreas, a peculiar circumscribed mass is present at the edge of one of the lobules, well within the substance of the gland. On examining the block from which this section was cut, the mass was found to measure 1.7 × 1.7 × 1.4 mm.

The mass is definitely encapsulated with fibrous tissue. There are no elastic fibers demonstrable in the capsule. The surrounding pancreatic tissue is definitely compressed by the mass which consists of cells similar in appearance to those of the islands of Langerhans. No ducts are connected with the tumor.

Under low magnification the tumor is seen to be composed of irregular masses and cords of cells, with a fibrous tissue stroma (Fig. 1). Viewed under high magnification, the tumor cells are rather small and poorly demarcated from one another. They tend to a cuboidal or rarely low cylindrical shape. Blood-filled sinusoids or lacunae are frequently in contact with the cell cords. The nuclei are vesicular with several chromatin masses. No mitotic figures are seen. The cytoplasm takes a pinkish stain with eosin-methylene blue. No zymogen granules are present. Owing to the type of fixation (Zenker's fluid with acetic acid) the presence or absence of $\alpha$- granules could not be determined, but with Bowie's stain, $\beta$-granules could be demonstrated.

CASE II. A–22–149 (Peter Bent Brigham Hospital). Male, 63 years old. Clinical diagnosis: Myocarditis with decompensation. Anatomic diagnosis: Chronic vascular myocarditis.

The pancreas is negative in gross. One of the slides for microscopic examination shows a rounded mass 1.3 × 1.1 mm. with a thin fibrous tissue capsule. The tissue shows moderate postmortem changes. Those acini near the mass are compressed. Islands of Langerhans are numerous in the section, and this, together with the size of the ducts, suggests that the tissue is from the tail of the pancreas.

The tumor mass consists of low cylindrical cells arranged in cords and irregular masses, with a delicate stroma. Many large capillaries are in contact with the epithelial elements. The cells have an acidophilic cytoplasm and rather large vesicular nuclei with a few prominent chromatin masses. No mitotic figures are present.

CASE III. U13–19 (Boston City Hospital). Clinical diagnoses: Pernicious anemia; syphilis. Anatomic diagnoses: (Primary anemia); hypostatic pneumonia.

The pancreas is negative in gross. Microscopic examination reveals a rounded encapsulated mass, 1.2 mm. in diameter, with moderate distortion of the surrounding parenchyma from its pressure. There is a moderate increase in fibrous tissue throughout the pancreas, with slight infiltration by mast cells and lymphocytes. The islands are negative.

The tumor is made up of irregular masses of cells embedded in a fibrous tissue stroma (Fig. 2). These cells closely resemble those of the islands. They are roughly cuboidal with indistinct cell bound-

aries. The cytoplasm is acidophilic, with only a few granules. The nuclei are large, rather clear, with prominent masses of chromatin. No mitotic figures are seen. Capillaries are in intimate contact with the cell masses. There is no suggestion of the presence of ducts.

CASE IV. A-09-101 (Boston City Hospital). Negress, 48 years old. Clinical diagnoses: Chronic nephritis and mitral stenosis. Anatomic diagnoses: Chronic nephritis and mitral stenosis.

On section of the pancreas, a sharply outlined reddish brown mass is noted at the junction of the head and body of the gland. This mass measures 9.0 × 6.5 × 6.0 mm. Under low power it is seen to be made up of cords and masses of cells supported by a rather delicate fibrous stroma. The blood supply is very abundant. The capsule is definite, varying from 200 to 800 microns in thickness. In the thicker portions, compressed pancreatic acini and one or two islands are included within the limits of the capsule. The surrounding pancreatic tissue is distorted and compressed.

Under high power these cells are seen to be very similar to the island cells themselves (Figs. 4 and 5). They are indefinite in outline, but tend to a cuboidal shape. Their cytoplasm is relatively clear and tends to be acidophilic. The nuclei are relatively clear with several large chromatin masses in them. Mitotic figures are absent. With eosin-methylene blue staining there is no evidence of zymogen granules. In places the cells form lacunae which appear to be filled with blood, and they are in intimate contact with capillaries throughout.

## DISCUSSION

That these abnormalities of the islands of Langerhans are much less rare than has been supposed is shown by the fact that three have been encountered at a single laboratory in routine microscopic examination of the pancreatic tissue removed at necropsy. If the pancreas were given less perfunctory attention by the average pathologist, this lesion would probably be found much more commonly.

Their occurrence has no relationship to other more prominent pathologic changes. In only one case (that of Heiberg [10]) was diabetes present, and here no connection between the two conditions is apparent. This lack of correlation with other lesions is rather against the interpretation of these tumors as hyperplastic islands, toward which Cecil [2] leans.

Size alone cannot be accepted as a criterion for these tumors. In Figs. 2 and 3 are shown an adenoma (Case III) and a very large island, respectively. The first was found in the pancreas of a female of 49 years dying of pernicious anemia and hypostatic pneumonia, while the second came from a negress of 54 years, who died of erysipelas. These two islands each measure 1.2 mm. in diameter, whereas the average normal island does not exceed 0.2 mm. In cases of diabetes, islands 1.5 mm. or more in diameter have been occasionally found.

In the two islands considered here, diabetes is not a possible cause of enlargement, nor is any other cause apparent. Fig. 3 is obviously that of an island normal except for its size. The arrangement and character of the cells and their relation to the surrounding tissue all suggest merely an overgrown island. But Fig. 2 (Case III) shows a different type of growth. Here the cells and their arrangement again suggest island tissue, but in addition there is a definite capsule and the surrounding tissue has obviously been compressed and distorted by the growth of the mass.

There is no characteristic age at which these tumors have been found, though most have been discovered in persons dying in the later years of life. These tumors are all slowly growing structures, and probably have existed for years before their presence happens to be detected through postmortem examination. They probably never give rise to trouble during life and have no clinical significance. It is rather interesting that tumors of the island cells, though rare, are nearly always benign, while tumors suggesting origin in the acinar cells in most cases are malignant. This variation would suggest a higher degree of differentiation in the island than in the acinar cell.

## Summary

1. Four cases of adenomas of the islands of Langerhans are presented, bringing the number of reported cases up to twenty.

2. These tumors are characterized by resemblance to the islands in arrangement of their cells and in appearance of the individual cell, by absence of mitotic figures, by the presence of a definite capsule and by compression of the adjacent tissue.

3. They are not so rare as the small number of reported cases would lead one to believe.

I am indebted to Dr. F. B. Mallory for permission to report the cases from the Boston City Hospital and to Dr. S. B. Wolbach for permission to report the case from the Peter Bent Brigham Hospital.

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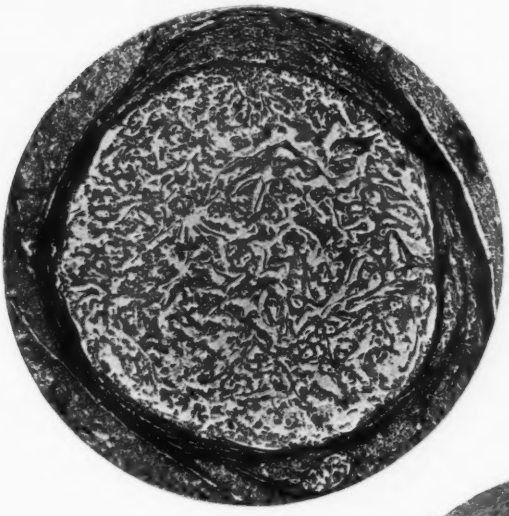
## DESCRIPTION OF PLATES

### PLATES 66–67

FIG. 1. Adenoma of island of Langerhans (Case I). x 40.
FIG. 2. Adenoma of island of Langerhans (Case III). x 40.
FIG. 3. Large island from normal pancreas. x 40.
FIG. 4. Adenoma of island of Langerhans (Case IV). x 400.
FIG. 5. Normal island from pancreas (Case IV). x 400.



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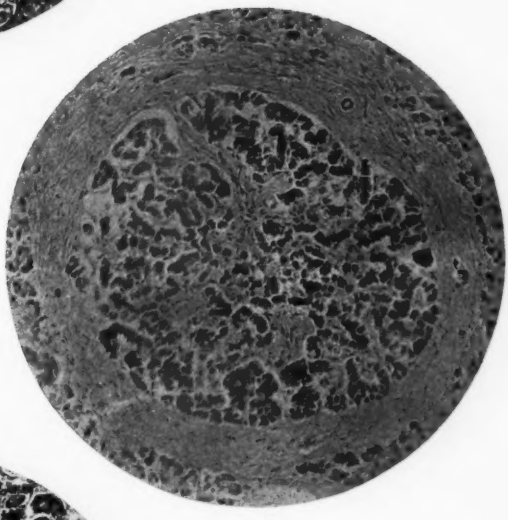

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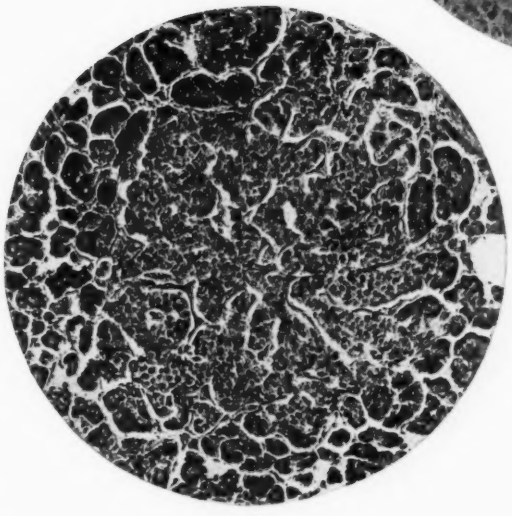
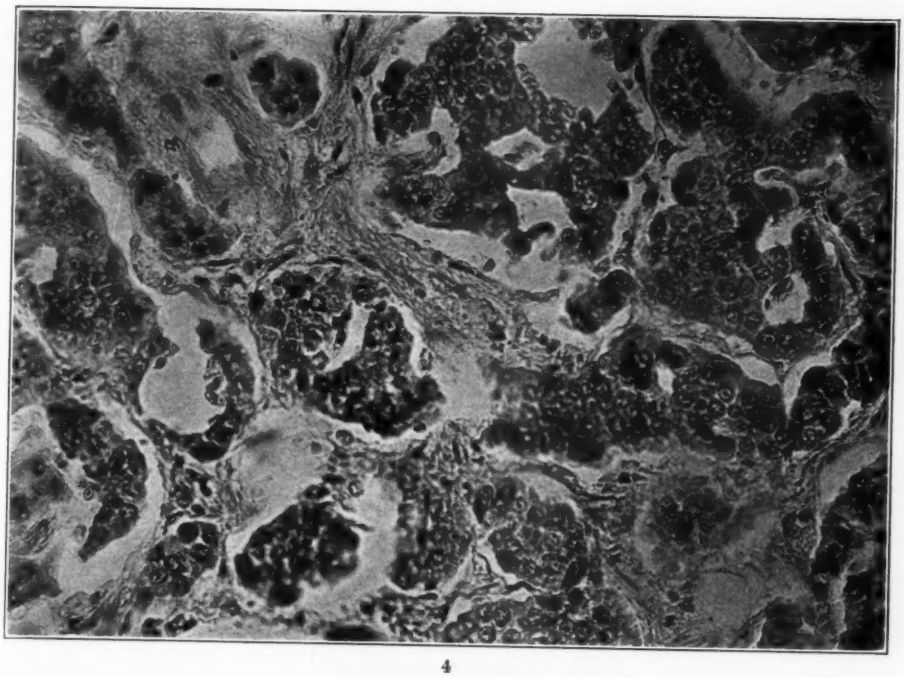


3

Warren

Adenomas of Islands of Langerhans



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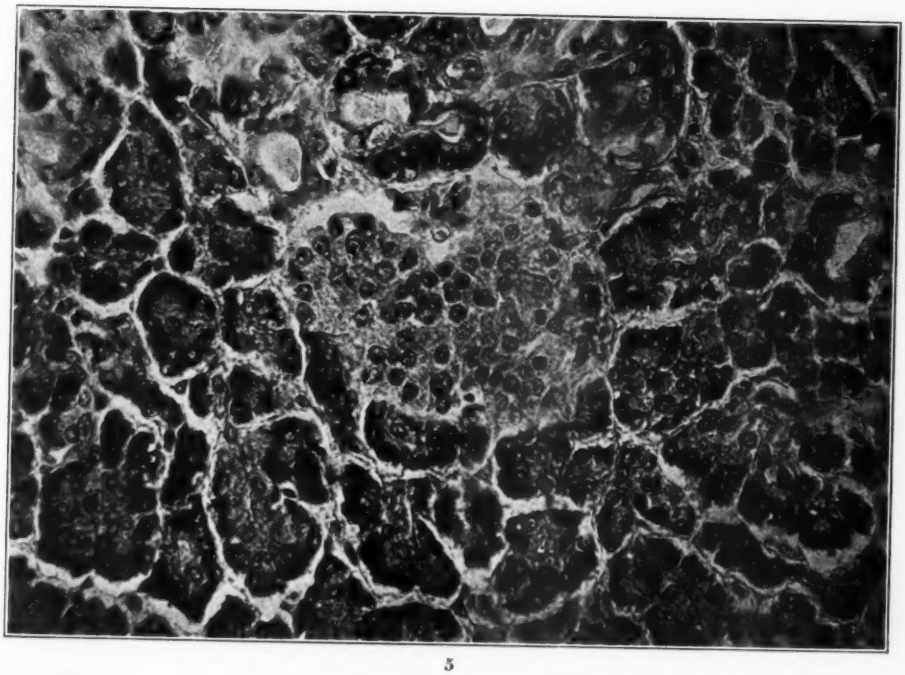

5

Warren

Adenomas of Islands of Langerhans